



**Host plant location, selection and preference
by the wheat bulb fly *Delia coarctata* Fall.
(Diptera: Anthomyiidae)**

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Abstract

Wheat Bulb Fly (*Delia coarctata* Fallén, *Diptera: Anthomyiidae*) is an important pest of winter wheat in the eastern half of Britain, and in northern and eastern Europe. There is one generation per year; eggs are laid in bare soil from late July to September. The eggs enter diapause which is broken after mid-January, when soil temperatures rise above 0°C. Neonate larvae must find a host plant and invade a tiller soon after hatching.

Winter wheat (*Triticum aestivum* L.) is the preferred cereal host, but other winter cereals and related grasses may also be attacked. All are annuals, except for the perennials couch grass (*Elytrigia repens* (L.) Nevski syn. *Elymus repens* (L.) Gould, *Agropyron repens* (L.) Beauv.) and perennial ryegrass (*Lolium perenne* L.). On wheat, high larval mortality occurs when neonate larvae fail to find a host, and when developing larvae kill their host plants. The geographical distribution and phenology of WBF are matched more closely with those of couch than with those of other hosts. These factors suggest that couch, and not wheat, is the preferred host. Aspects of this hypothesis were tested in the laboratory, glasshouse and field.

In choice test bioassays neonate larvae chose couch seedlings and their exudates over wheat seedlings and their exudates, and couch rhizome exudates over controls. Couch seedling exudates had attractant properties, whereas wheat exudates had attractant and arrestant properties, when compared with controls. The larvae were photophobic and positively geotactic.

In a pot trial, symptoms of infestation appeared earlier in couch than in wheat. Attacked plants responded by producing extra shoots, which were also killed by larvae; this response was greater in couch than in wheat. After 5 weeks, infested plants suffered a relative reduction in number of shoots, but uninfested neighbouring plants, especially wheat, compensated for this by producing more shoots themselves.

Larvae raised on couch emerged as adults earlier than those raised on wheat. They thus develop more rapidly, and use more resources, on couch than on wheat, i.e they are better adapted to couch as a food source. Earlier eclosion would allow adults to make better use of favourable weather conditions, and to live longer, mate more often, and produce more eggs. Older eggs developed more rapidly to adulthood.

In laboratory and field adult WBF preferred to rest on couch than on wheat. Buried couch rhizomes did not encourage WBF oviposition in the laboratory or the field.

These findings support the hypothesis that couch is the preferred host of WBF, provide a partial explanation of high larval mortalities on wheat, and suggest that attractants isolated from couch and arrestants isolated from wheat could be used in WBF control programmes. The ecological implications of a preference for couch as a host are discussed.

Dedication

I would like to dedicate this thesis to the women in my life:

To my late great-aunt Kathleen Keswick and my mother Sally, without whose generosity this would not have been possible;

To my wife Jane, and our children Katharine and Lizzie, whose love, patience and emotional support have kept me sane.

Declaration

I hereby declare that this thesis is my own composition and that all the work reported was carried out by myself except where clearly and specifically acknowledged.

Charles Marriott.

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Chapter 1

General introduction

Chapter 1. General introduction

Wheat bulb fly (*Delia coarctata* (Fallén), Diptera: Anthomyiidae) (WBF) is one of the most serious insect pests of winter wheat (*Triticum aestivum* L.) in Britain (Gratwick, 1992). It is prevalent in eastern counties, from Kent in the south to Tayside in the north. The pest is widely found in central and northern Europe (Long, 1960a), extending into southern Russia (Commonwealth Institute of Entomology, 1987). It favours a warm, dry, continental type of climate, with annual rainfall of less than 840mm (Thomas, 1948), and at least two months of temperatures below 10°C, although it can tolerate winter temperatures as low as -20°C (Way, 1959).

Damage varies considerably from year to year. Agricultural Development and Advisory Service (ADAS) records for the past 40 years show that in eastern counties of England where the pest is established, the proportion of wheat fields with economically damaging numbers of eggs may vary annually between approximately 5 and 50% (Young & Cochrane, 1993). Between 1984 and 1993, the estimated financial value of yield losses caused by WBF in England ranged from £5.8 million to £33 million, but the estimated net benefit gained from chemical control ranged from £2.1 million to £14.8 million (Young & Ellis, 1996). Oakley and Young (2000) estimate that between 1984 and 1999, the proportion of wheat crops at risk varied between 3 and 44%. Due to current high costs and low grain prices, many treatment strategies may not recover the cost of treatment in years of low incidence (Oakley & Young, 2000).

Damage is inflicted by WBF larvae killing the young central shoots of susceptible grasses or cereals between hatching in late January and pupation in April or May. Variation in WBF damage is due mainly to climatic factors, operating directly on the WBF population, or indirectly through the growth stage of susceptible crops at the time of attack. Thus, hot, dry conditions during egg-laying in July and August reduce the number of WBF eggs laid, and WBF damage tends to be worse in cold

winters, due to slower crop development and a reduction in the capacity of the crop to compensate for loss of plants or tillers during the winter (Young & Ellis, 1996).

1.1. Life cycle of the wheat bulb fly

Wheat bulb fly has just one generation per year, with eggs laid in bare soil, before wheat is sown, from late July to September. The greatest risk of attack occurs in wheat sown after (in descending order) fallow, potatoes, vining peas, sugar beet and oilseed rape (Young & Ellis, 1996). Early harvesting and low, open crop canopies encourage wheat bulb fly oviposition. McKinlay and Franklin (1980) found that more eggs were laid on potato ridges than in furrows, and ascribed this effect to the greater apparency of the ridges. The eggs enter an obligatory diapause which is only broken after mid-January, when soil temperatures are above freezing (McKinlay, 1980).

Neonate larvae must find a host plant and invade a tiller soon after hatching. Each larva enters the centre of a shoot just above the meristem; this results in the death of the central rolled leaf, giving the characteristic symptom of “deadhearts” (Gough, 1946). A third-instar larva, deadhearts, and field-scale WBF damage, are illustrated in Figs. 1.2a, 1.2b, and 1.2c, respectively, on page 5. Wheat is the preferred cereal host. Barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), triticale and various grasses may also be attacked, but oats (*Avena* spp.) are virtually immune (Gemmell, 1927). If the wheat plants are able to produce more tillers, due to early sowing, favourable growing conditions, or robust varietal characteristics, then the crop may recover from wheat bulb fly attack (Bardner, 1968; Young, 1992).

If the larva kills the tiller it has invaded, or the whole plant, the second or third instar may migrate to a new tiller or plant (Gough, 1947). The larvae mature and grow through three instars, then leave the plant to pupate between mid-April and mid-May. The pupae rest beneath the soil surface near the plant, and the adults emerge in June.

They remain at the emergence site for about three weeks to feed, mature, and mate, after which the females fly in search of a suitable oviposition site.

The entire life cycle is illustrated in Fig. 1.1, below.

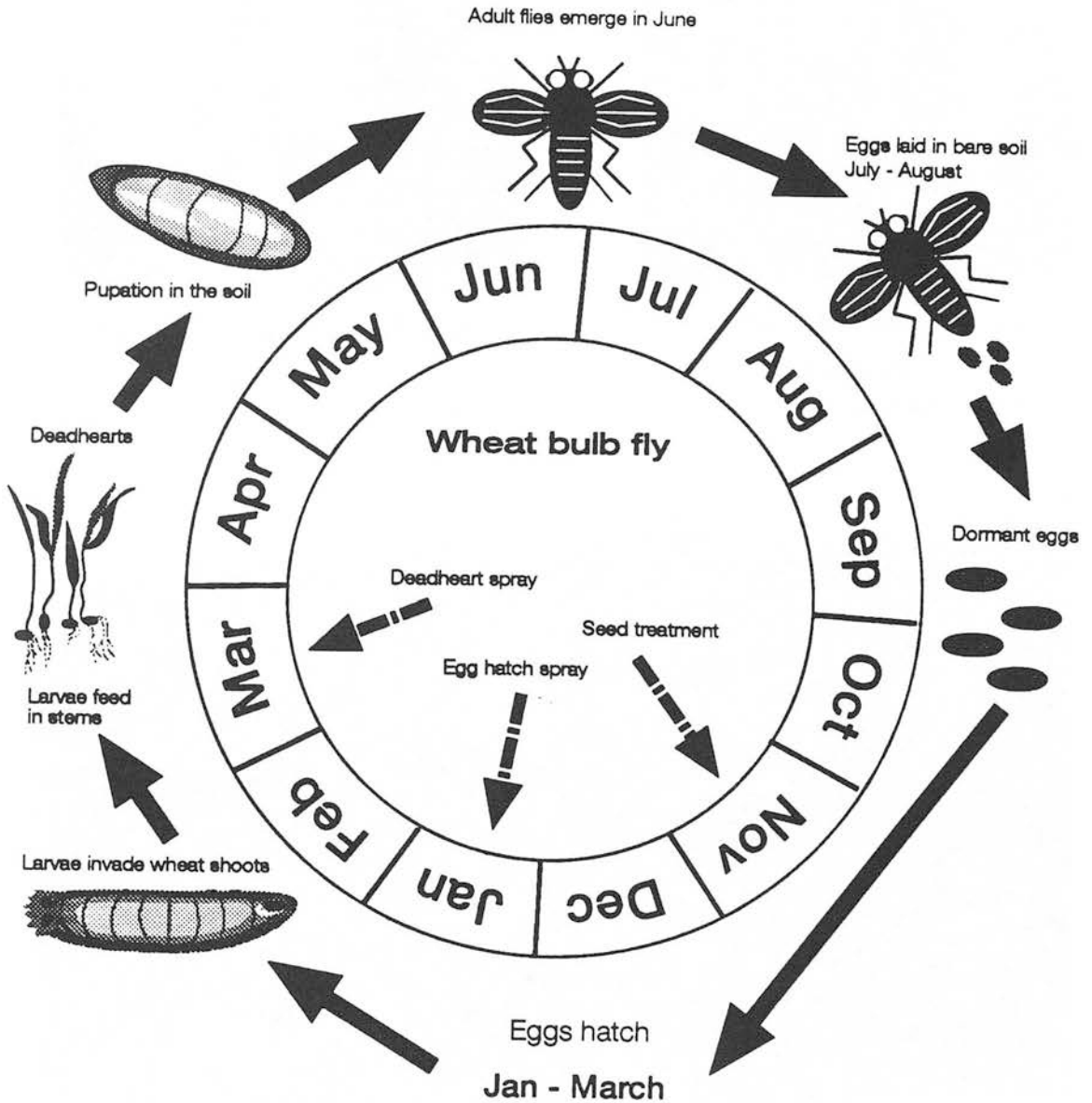


Fig. 1.1. Life history of the wheat bulb fly
From Young and Ellis (1996) © HGCA



Fig. 1.2a. Third instar WBF larva in wheat shoot

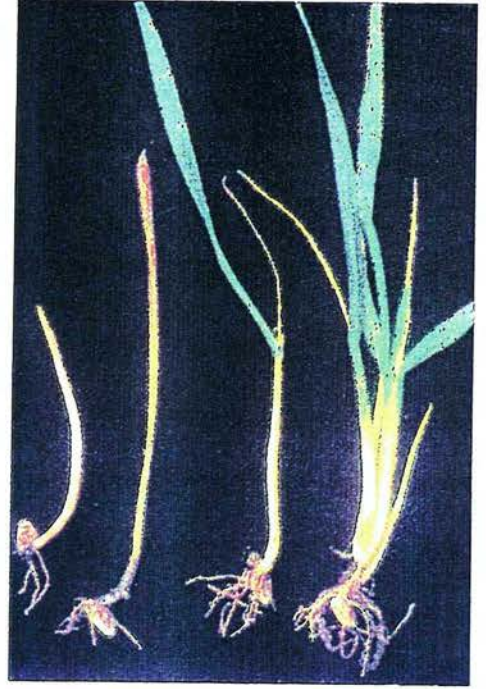


Fig. 1.2b. WBF damage to wheat seedlings



Fig. 1.2c. WBF damage to wheat, and chemical control

Left: Pre-drilling treatment with fonofos granules

Right: Untreated

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1.2. Natural mortality of wheat bulb fly

1.2.1. Egg mortality

Ryan (1973a) found in single-choice laboratory bioassays that WBF eggs were eaten by the carabid beetles *Agonum dorsale* Pont., *Trechus quadristriatus* Schr., and *Clivinia fossor* L. In an earlier study (1967) he found that predation of WBF eggs between August and February was very variable, and never more than 20%. Of this predation loss, 50% occurred between 17 August and 7 September, and 90% could be attributed to carabids. In pitfall traps Ryan (1973a) found more than 5 times as many carabids in the border of a wheat crop than in nearby fallow. In Ryan's field studies (1973a), excluding predators from plots where eggs were found did not affect egg mortality, although in a similar study Jones (1975) found that 50-67% of eggs disappeared where known numbers were placed in the soil and controls were protected from predation. Most of the eggs in the latter study disappeared before the end of October, when *T. quadristriatus* was abundant. Thus, although carabids can eat WBF eggs, their effect on egg numbers in the field is variable and uncertain.

Raw (1967) found that 20% of eggs laid were not viable. Ryan (1973a), however, found that only 1% of WBF eggs were diseased, and only some 2-14% were sterile.

1.2.2. Larval mortality

Young (1992) found that mortality of eggs and neonate larvae before plant invasion can be more than 90%. Gough (1946) estimated that 56-81% of larvae died between hatching and plant invasion. Long (1960b) also found very high mortalities of neonate larvae. Such high mortalities suggest that neonate WBF larvae experience some difficulty in finding wheat shoots even in the artificial situation of a dense, evenly spaced population of young, susceptible seedlings. Or in other words, even the most suitable wheat seedlings in favourable conditions are not sufficiently attractive to neonate larvae to prevent them dying in large numbers before they find a host. Like Young (1992), Long (1960b) found neonate mortalities were highest in

peaty loam (98%) compared with sandy loam (73%). This may be due to the physical characteristics of the soil impeding larval movement.

Ryan (1973b), while supporting these figures, found that a further 67-81% of larvae died after invading plants. Many plants were killed and surviving plants compensated by producing shoots which were, he hypothesised, too small for the growing larvae. First and second instar larvae consume, on average, one shoot between them, but the third instar consumes a further four shoots (Gough, 1947).

Thus, in wheat, the two most significant factors affecting larval mortality, and indeed the mortality of a whole generation of WBF, are the failure of neonate larvae to find hosts, and the destruction of a suitable food supply by developing larvae. These factors are reflected in Raw's (1967) finding of a relationship between neonate larval mortality and the number of shoots available to them for food. Furthermore, Ryan (1973b) demonstrated that larval survival to pupation was related both to the number of shoots per hatching larva, and the number of shoots per feeding larva.

These high mortalities of neonate and later instar larvae suggest that wheat is a less than ideal food plant, and may not be the insect's preferred or natural host. It should be noted that all these figures relate to larval mortality in wheat, usually in the highly unnatural situation of ploughing, cultivating and sowing the soil in which WBF eggs have been laid. There have been no studies of WBF larval mortality in any other of its known host plants.

Ryan (1973b) and Jones (1975) concluded that predation had very little effect on larval mortality, since for most of the time the larva is adequately protected inside the host plant.

1.2.3. Pupal mortality

Natural enemies are a much more important cause of mortality in WBF pupae than in their eggs or larvae. Ryan (1975) found pupal losses ascribed to predators of between 15 and 34% in four populations. These predators were probably carabid beetles. A further 0.5-5.8% of pupae were killed by parasitic Hymenoptera, mostly *Phygadeuon trichops* Thomp., with 3 cases of parasitism by *Trichopria* sp.

1.2.4. Adult mortality

Fungal parasites, mostly *Entomophthora* spp., can attack adult WBF, and in some years can cause significant mortality. Wilding and Lauckner (1974) found that the proportion of flies infected increased with the number of flies emerging that year. In a field study at Rothamsted in 1970, two thirds of emerging females were killed by *Entomophthora* before they could lay eggs, indicating that such parasites may be very important in the regulation of WBF populations. Wilding (1969) also reported attacks on adult WBF by the fungus *Strongwellisia castrans* Batko & Weiser.

The effects of predators on adult WBF are little reported. Jones (1975) observed the predatory fly *Empis livida* L. and dung flies (*Scathophaga* spp.) eating WBF. Other general predators may include birds and spiders.

1.3. Control of wheat bulb fly

Earlier sowing, preceding crops which discourage egg-laying, and cultivating a fine tilth before or after egg-laying can reduce the risk of WBF attack. However, the main controls are chemical, using organophosphates as a seed treatment, seedbed spray, or egg-hatch spray (Young, 1992), or the synthetic pyrethroid tefluthrin as a seed treatment (Frost, Elsworth & Moran, 1994). If these measures are not taken, or if they fail, one or more dimethoate sprays may be applied when deadhearts appear. Chemical controls are costly and may fail; declining cereal prices in recent years have further reduced their economic benefits (Oakley & Young, 2000). Furthermore,

concern over the toxicity (Marrs, 2000) and environmental safety (Burn, 2000) of organophosphates may threaten their continued approval and use (Young & Ellis, 1996). There is also evidence (Obadofin and Finlayson, 1977; Vickerman, 1992) that organophosphates, by killing predatory carabid beetles, may encourage crop pests.

A number of insecticides formerly used on WBF have already been withdrawn, or their manufacturers have failed to re-register them. There is every reason to suppose that the list of chemical insecticides approved for use on these pests will be further restricted (Young & Ellis, 1996).

Some progress towards integrated pest management has been made, particularly in the use of forecasts to predict pest outbreaks (Young & Ellis, 1996). Better forecasting has allowed some growers to forgo chemical controls in low-risk situations, or to reduce the number of chemical applications.

Researchers have not yet identified durable resistance traits to wheat bulb fly (Young & Ellis, 1996). However, varieties with greater tillering capacity are more able to withstand WBF attack (Raw, 1967).

The main non-chemical methods of controlling WBF are still cultural. Earlier sowing can greatly reduce the risk of damage, and adjusting rotations to avoid encouraging the pests can also be effective (Gratwick, 1992; Young & Ellis, 1996).

A number of predators, parasites and pathogens of WBF have been identified, but so far little work has been done to develop them as biological control agents (Young & Ellis, 1996).

1.4. Host range of wheat bulb fly

The first serious study of WBF (Ormerod, 1890), suggested that couch grass (*Elytrigia repens* (L.) Nevski syn. *Elymus repens* (L.) Gould, *Agropyron repens* (L.)

Beauv.) was also a host. Gemmill (1927) successfully reared WBF larvae to the pupal stage on barley, rye, *Dactylis glomerata* L. and couch grass. He noted larvae entering and feeding on young couch rhizomes as well as actual shoots, and found they could bore through the nodes on the rhizomes. Indeed, Shaw and White (1969) describe a case of WBF damaging spring barley in areas of a field which had a heavy infestation of couch.

In arable land, Gough (1946) found WBF larvae in *Poa trivialis* L., *P. annua* L., *Agrostis nigra* With. and couch, while in the laboratory they also infested *Phleum pratense* L. and *Lolium perenne* L.

Stokes (1955) successfully bred WBF on the grasses *Agrostis tenuis* Sibth., *Festuca pratensis* Huds., *Hordeum murinum* L., and *Poa pratensis* L.. Flies were also bred from four non-British plants related to wheat, *Aegilops ovata* L., *Triticum dicoccum* Schubler, *T. turgidum* L., and *T. turgidum* L var. *dicoccoides*. Larvae were found in *D. glomerata*, *T. spelta* L. and *T. turgidum* L. var. *durum*, while typical damage was found in barley, *Festuca rubra* L, *L. perenne*, *P. pratense*, *P. annua* and *T. compactum* Host (Stokes, 1955).

Raw and Stokes (1958) found WBF larvae in field samples of *D. glomerata* and *L. perenne*, and two other species, *P. annua* and *E. repens*, showed damage characteristic of WBF. In a series of unreplicated pot trials, neonate larvae were placed in the centre of pots in which wheat and another host plant were grown in approximately equal amounts. The numbers of larvae subsequently found in the test plants, relative to the numbers found in wheat, were as follows (wheat = 100):

Table 1.1. Larval infestation of WBF hosts, relative to wheat

<i>Elytrigia repens</i>	Barley	<i>Agrostis tenuis</i>	Rye	<i>Festuca pratensis</i>	<i>Poa pratensis</i>
133	83	57	26	23	18

Thus, although WBF larvae have been found on a wide range of cereals and related grasses, they have only been shown to survive to pupae on wheat, barley, rye, couch grass, *D. glomerata*, *Agrostis tenuis*, *Festuca pratensis*, *Hordeum murinum*, and *Poa pratensis*. Of these, couch appears the most preferable.

Furthermore, although the taxonomy of the tribe Triticeae is much revised and debated, of the various grasses mentioned above, only the genus *Elytrigia*, or its earlier synonyms *Elymus* and *Agropyron*, is consistently included along with *Triticum*, *Hordeum* and *Secale*. None of the other host genera of WBF has ever been included in the Triticeae (see, for instance, Dewey, 1984). Couch is very closely related to wheat and can even be artificially crossed with it (Harlan, de Wet, & Price, 1973; Franke, Nestrowicz, Senula & Staat, 1992).

1.5. Geographical distribution of wheat bulb fly and its hosts

The current world-wide distribution of WBF is shown in Fig. 1.3, overleaf (Commonwealth Institute of Entomology, 1987). This coincides broadly with Eurasian areas having a humid continental climate with cold winters, and the eastern part of Europe's maritime climate, as described by Money (1988). Within these areas, it appears to be limited to within the 840mm rainfall isohyet (Thomas, 1948). WBF has also occasionally been reported outside these areas, although rarely as a pest of wheat. McAlpine and Slight (1981) found WBF in eastern Canada and the USA, associated with couch and not wheat; they concluded that the insect had been introduced from Europe. Ackland (pers. comm.) has found the insect in many non-arable habitats, such as Culbin Sands near Inverness, and Abisko National Park within the Arctic Circle in Sweden. WBF has been reported in Tunisia and Iraq, but not for at least 36 years (Gentry, 1965).

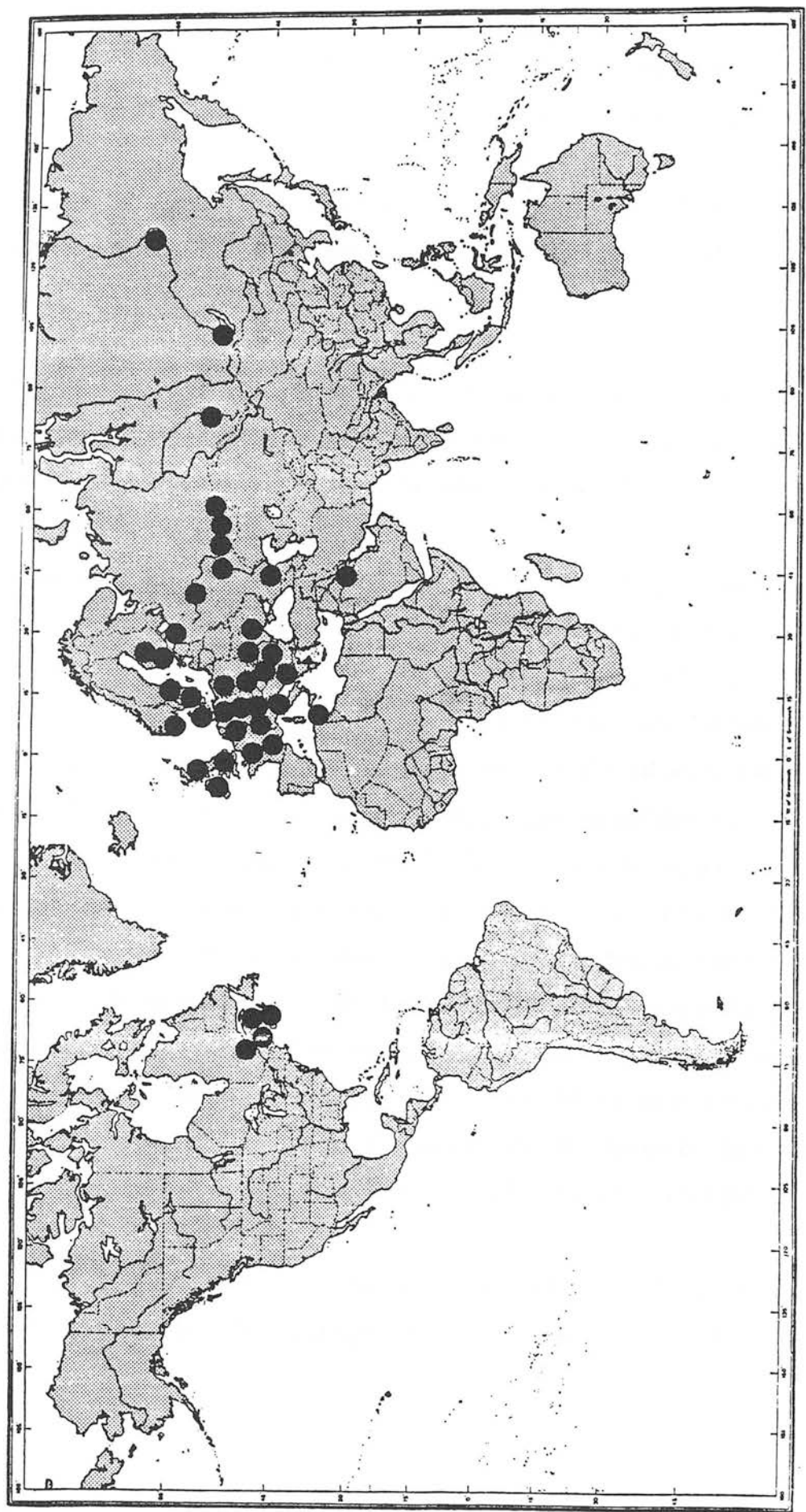


Fig. 1.3. Geographical distribution of wheat bulb fly, *Delia coarctata*. Commonwealth Institute of Entomology, 1987.

Evans and Hughes (1996), and Evans, Hughes and Aspinall (1996), suggest that, according to their work on climatic models, much wider areas of arable land in England and Scotland are climatically at risk, but the pest's distribution is limited by cropping patterns and the poor flying ability of adults. The increased use of summer fallows in the management of set-aside may also cause localised increases in wheat bulb fly populations in areas currently considered to be at marginal risk (Young & Ellis, 1996).

WBF's known cereal hosts, wheat, barley and rye, all evolved and developed in the Middle East or around the Mediterranean (Smartt and Simmonds, 1995). It is therefore highly unlikely that they are the ancestral hosts of WBF.

The ancestors of WBF's grass hosts are thought to have been perennial species of forests and forest margins of the Eurasian temperate zone, and to have migrated northward since the last ice age (Scholz, 1975). Later they polarised into species favouring wet or dry areas. In moister areas, *Poa*, *Festuca*, *Lolium* and *Dactylis* were significant, and *Agrostis* also occurred. In the drier continental areas, most suited to WBF, *Elymus/Agropyron* (presumably including *Elytrigia*) and *Bromus* (not a host of WBF) were most significant (Smith, 1995). Couch is naturally distributed, according to Palmer and Sagar (1963), throughout Europe from the Arctic into northern temperate Africa, southwest temperate Asia, western and central parts of the former USSR, and northwest India. Holm, Plucknett, Pancho and Herberger (1977) describe couch as "present in all the major agricultural areas of the north temperate zone", and add that "Some workers have speculated that it cannot succeed without a cold dormant period. Those who have worked with the species are impressed with the vigorous vegetative growth which seems to follow the cold winter period."

Thus, of the various hosts in which WBF has been found, the geographical and climatic distribution of *Elytrigia repens* most closely matches that of the insect.

1.6. Phenology of wheat bulb fly and its hosts

In natural conditions, WBF's cereal hosts, and indeed any other of its annual grass hosts, would be expected to shed seeds in mid- to late summer (June to August), the majority of which would then germinate in the early autumn (August to October). Only a small fraction of the resulting seedlings would be small enough to be susceptible to WBF attack when the larvae hatch in late January or early February. Even suitable seedlings are liable to be killed by developing larvae, which then have to find another suitable host (Ryan, 1973b).

Couch, on the other hand, starts to produce aerial shoots from the apical buds of young rhizomes in early autumn. These shoots grow very slowly during the winter, and by early spring will still have only 2 or 3 leaves (Palmer, 1958). Thus, when WBF larvae hatch in late January and early February, the couch shoots are most susceptible to attack. In March and April, the couch shoots rapidly produce new leaves, and previously dormant buds form tillers or new rhizomes. Damage to shoots with two or more leaves encourages growth of tillers, and damage to apical shoots of rhizomes encourages shoot development of the nearest lateral bud (McIntyre, 1970). The developing WBF larva is thus assured of a constant supply of suitable feeding material.

All of WBF's known hosts produce flowering heads in June or July when adult flies emerge, rest on grass stems, and feed on the saprophytic micro-organisms found on the ears of grasses and cereals (Jones, 1970a).

Female WBF lay their eggs in apparently bare soil between the end of July and mid-September, often before their cereal host plants are even sown. However, at this time of year couch plants, especially in relatively open cultivated situations, produce abundant rhizomes which may spread laterally for up to 2m (Palmer, 1958). The response of gravid WBF to buried couch rhizomes, or to young aerial shoots of couch, has never been investigated.

1.7. Aims of the research

The aims of the current research were to compare the relationships of WBF with wheat and couch at all the active stages of the insect's life cycle. The main hypothesis is that couch, and not wheat, is the natural host of WBF. Although it is probably impossible to test this central hypothesis, a number of sub-hypotheses can be developed and tested. The implications for the ecology, biology and control of the insect will be discussed.

Chapter 2

General materials and methods

Chapter 2. General materials and methods

2.1. Introduction

Laboratory and glasshouse experiments used wheat bulb flies (WBF) or their larvae raised in the laboratory. Large-scale rearing is hampered by the obligate 100-day diapause of eggs (Way, 1959) and the feeding requirements of adults (Bardner & Kenten, 1957), which are very demanding of labour. Thus, although cold storage of eggs allows adults to be bred throughout the year, the duration of the life cycle cannot be reduced below 15 weeks (Jones and Moore, 1978). Jones and Moore (1978) obtained about 80,000 eggs from about 4000 field-collected adult flies. When they fed larvae on wheat plants, 55-70% of those hatching from eggs became flies.

2.2. Sources of insects and plants

WBF eggs were collected in September 1998 and September 1999 from soil on the tops of potato ridges at three neighbouring farms; Highfield, Sydserf and Rockville Farms, near North Berwick, East Lothian. All three farms had a history of WBF damage. The eggs, and any found in the Scottish Agricultural College's regular sampling and monitoring work, were removed from the soil by sieving and flotation, as described in SAC Standard Operating Procedure CER 020 (See Appendix 1). In 1998, recovered eggs were placed between discs of nylon mesh in sealed 9cm diameter Petri dishes of moist sand, and stored at 4°C for at least 16 weeks. The following year, sand was replaced with moist vermiculite, and the eggs were stored for two months at 15°C, then at least two months at 4°C.

Collection of soil and removal of WBF eggs from the soil are very time-consuming processes which place a limit on the number of eggs available for laboratory rearing and experiments; 1279 eggs were extracted from approximately 3335kg soil in 1998, and 3446 eggs from approximately 725kg in 1999.

Couch (*Elymus repens*) rhizomes were collected from a heavily infested field (Cow Loan) at Boghall Farm, Midlothian, on 26th January 1999, and stored at 4°C in black plastic bin-liners. When needed for bioassays or rearing, shoots which had not passed the one-leaf stage were excised from the rhizomes, together with their associated node and roots, and no more than 1cm of rhizome. If necessary, rhizomes were grown in the glasshouse in peat until enough shoots of the right size had been produced. In 1999, these shoots were used for all relevant laboratory and glasshouse experiments, and for rearing wheat bulb fly. Rhizomes produced by the rearing plants were used for experimental work in 2000.

Couch seeds used for laboratory experimental work and for rearing in 2000 were supplied by Herbiseed Ltd, Wokingham.

Wheat seeds used for laboratory and glasshouse experimental work, and for rearing wheat bulb fly, cv Mercia, were harvested from untreated plots on an SAC trial (Spotsmains WW96 ES 3U).

2.3. Laboratory rearing of wheat bulb fly

WBF were reared using the feeding and climatic conditions developed by Jones and Moore (1978), but with different cage designs, as detailed below. Due to the limited numbers of eggs available, experimental insects were also used for rearing. Thus neonate larvae which had survived bioassays were used for experiments on larval development, and adult flies emerging from larval development experiments were used for adult plant preference and oviposition bioassays. As a result, there were insufficient eggs laid to rear a second generation; hence the repeated collection of eggs from the field in September 1999.

2.3.1. Larval and pupal development

WBF eggs from cold storage were rinsed briefly in 1% NaOCl, then rinsed thoroughly in sterile distilled water, and placed in Petri dishes between two 9cm circles of black filter paper (Schleicher and Schuell, Dassel, Germany), moistened

with sterile distilled water. These Petri dishes were kept moist at 15°C and checked daily for newly-hatched larvae.

Neonate larvae were transferred directly, or later that day after bioassays, to rearing plants. In 1999, these were week-old wheat seedlings, or couch shoots derived from rhizomes, in 20cm pots of compost. One larva was placed beside each of ten plants in a pot of 20. The pots were placed in controlled climate cabinets at $13 \pm 2^\circ\text{C}$, 80% RH, 10 hours daylight, and watered at the base twice weekly. After 8 weeks, each pot was transferred to a glasshouse at ambient temperatures ($23 \pm 5^\circ\text{C}$), and covered with a perspex tube 50cm high. The pots were checked daily for newly emerged adult flies. To allow removal of adult flies, a stocking with the toe removed was stretched over the top of each perspex tube.

In 2000, WBF larvae were added to couch or wheat seedlings approximately one week after shoot emergence. Two hundred untreated wheat seeds were sown 2cm deep in John Innes No. 3 compost over gravel in plastic crates 30 x 55 x 38cm deep, with drainage holes drilled in the bottom, and placed in a growth room at a constant 15°C, with a 12hr day. After 2 weeks most of the seedlings had emerged.

Four hundred couch seeds were sown just below the surface of compost in identical crates. They were left in a Fison's growth cabinet at 10°C/12hr nights, 25°C/12hr days for 3 weeks, by which time most of the seedlings had emerged and were about to produce their first leaves. All crates of compost were initially soaked with water, then watered with an overhead spray twice weekly.

After addition of WBF larvae, all crates were kept in the 15°C growth room for 3 weeks, then transferred to cages in a growth room kept at a constant 20°C for a 12 hour day. Each crate was kept in a nylon mesh cage 45cm wide x 70cm x 1m high. The front, narrow side was fastened with Velcro™, and included a 12 cm hole closed with a stocking, allowing easy access to remove adult flies with an aspirator.

Hopkins (1917) hypothesised that insect larval experience of host plant could affect adult host plant and oviposition preference. However, this “Hopkins host-selection principle” has yet to be conclusively demonstrated, and many authors who have sought a transfer of larval feeding experience to the adult stage have failed to find such an effect (Szentesi & Jermy, 1990). Conversely, several apparent cases of Hopkin’s host-selection principle, for instance in *Drosophila melanogaster* Meigen (Barron & Corbet, 1999), have proved, on closer examination, to be examples of early adult, rather than larval, conditioning. It is not known whether WBF is similarly affected, but these issues are addressed in Chapters 6 and 7.

2.3.2. Adult eclosion and rearing

Adults emerging from the larval rearing pots or crates were transferred with the aid of an aspirator to one of two separate adult rearing cages depending upon the larval host plant. In 1999, these cages were 60 x 60 x 120 cm high with a perspex front, one solid wooden side, and nylon mesh on the roof and the two remaining sides. They were kept in the glasshouse at ambient temperature ($23 \pm 5^{\circ}\text{C}$). Around the sides of the cage were eight 12cm plant pots filled with compost, four containing a single wheat plant, and four containing a single couch plant, arranged alternately. In the centre of each cage a perspex shelf was balanced on the pots, and on this were four 9cm Petri dishes. These contained, respectively, sterile distilled water, 1g milk powder and 1g yeast powder in 10ml water, citrated sheep’s blood (donated by Diagnostics Scotland) diluted to 50% with water, and a 10% solution of honey in water, as recommended by Jones and Moore (1978). In each Petri dish the liquid was absorbed by a dental wick (Kent Dental Supplies) or, in the case of the water, cotton wool. These dishes were changed twice a week, and 5ml sterile distilled water was added to each on intervening days.

In 2000, the cages were identical to the larval rearing cages, except the roofs were shaded with cardboard. The cages were kept in the same growth room as the larval rearing cages. Each contained one plant of the same species on which the larvae had been reared, in a 20cm pot of compost, standing in a dish of water. On top of each

pot was a perspex shelf in two halves which fitted around the base of the plant. Adult flies were fed and watered as in 1999, except the food was presented in 4cm Petri dishes on the perspex shelf. In both years, adult females were transferred to oviposition cages when they appeared gravid, with swollen abdomens.

2.3.3. Oviposition

Climatic conditions and feeding for gravid females were as suggested by Jones and Moore (1978), but since their numbers were low, the cages, illustrated in Fig. 2.1 overleaf, were similar to those used by Bardner and Kenten (1957).

In 1999, oviposition cages consisted of a glass lantern cover with a top and bottom made from 9cm Petri dishes. The top had four slots cut in it, in which rested dental wicks soaked in food or water as described above. These were moistened daily and replaced twice a week. A Petri dish lid was placed over the wicks to prevent them drying out. The bottom of the glass rested on a circle of perforated galvanised zinc from a dessicator, which in turn rested on a circle of 2mm wire raising the zinc above a circle of moist black filter paper in a 9cm Petri dish. The filter paper was checked daily for freshly laid eggs. The whole apparatus was fastened with a large rubber band, and kept in a controlled climate cabinet at 20°C, 12h daylight.

The lantern glass oviposition cages were difficult to handle, resulting in damage or escape of a number of flies. Furthermore, ventilation was poor, and the different types of food often came into contact, encouraging putrefaction. Despite being covered by a Petri dish lid, the wicks often dried out. In 2000, a new design of oviposition cage was used; however, since these were used only for experimental purposes, these are described fully in Chapter 7.

There were too few eggs laid in 1999 to rear a second generation. However, in 2000 nearly 300 eggs were laid, despite all the couch-reared adults, and many of the wheat-reared flies, dying due to over-heating of the growth room.

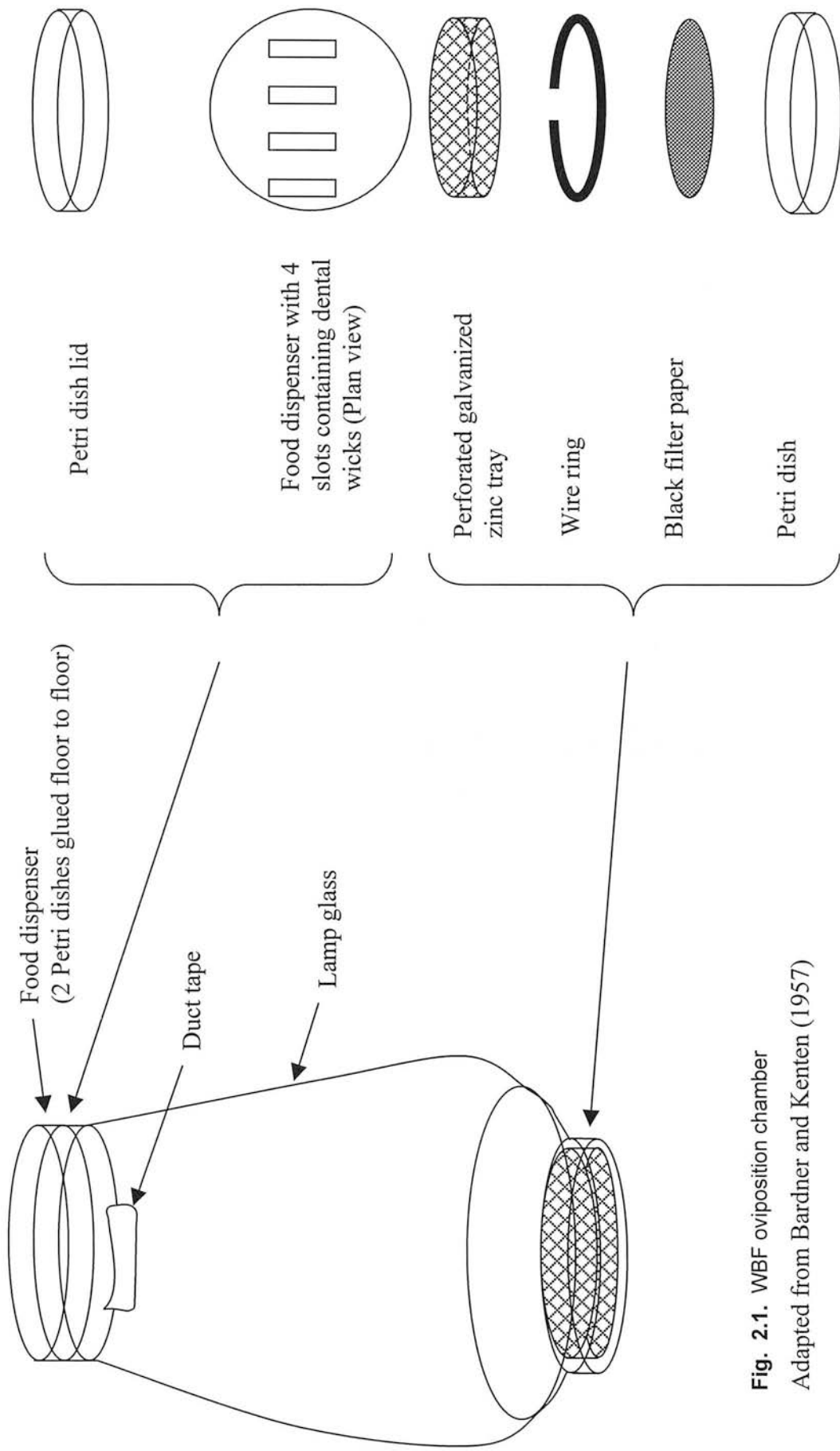


Fig. 2.1. WBF oviposition chamber
Adapted from Bardner and Kenten (1957)

Chapter 3

Host plant choice and location by wheat bulb fly larvae

Chapter 3. Host plant choice and location by wheat bulb fly larvae

3.1. Introduction

3.1.1. Host plant location by phytophagous insects

It has long been established that many specialist phytophagous insects find and select their hosts by responding to chemicals produced by plants (Dethier, 1947). Semiochemicals can be defined as “chemicals that mediate interactions between organisms” (Law and Regnier, 1971, quoted in Nordlund, 1981). They play a particularly important rôle in food location by soil-dwelling insects, where visual cues are not an option.

Semiochemicals produced by the plant may be primary metabolites, which are usually simple molecules such as sugars, carbon dioxide, or amino acids that play an essential role in the plant’s metabolism, or secondary metabolites, which are more complex and have no recognised rôle in plant metabolism. Primary metabolites are mostly very common products of a wide range of plants. No two plant species have the same profiles of secondary plant metabolites, so many species may be identified by their chemistry. Many plant secondary metabolites are restricted to particular taxa or even species (Bernays and Chapman, 1994).

Semiochemicals involved in finding and feeding on host plants fall into several categories: arrestants, which cause the insect to aggregate or to slow its movement in one direction; attractants, which cause the insect to make oriented movements towards their source; and feeding stimulants (phagostimulants), which elicit feeding. Conversely, deterrents inhibit feeding and repellents cause insects to make oriented movements away from their source (Nordlund, 1981). Košťál (1992) found that several “green-leaf volatile” compounds were repellent to larvae of the cabbage root fly (*Delia radicum* L.).

Jones and Coaker (1978), reviewing known chemical attractants to insects feeding below ground, found that polyphagous insects responded only to primary plant metabolites, whereas mono- or oligophagous insects might respond to primary and/or secondary metabolites specific to their food plants. Indeed, some secondary plant metabolites, such as the glucosinolates produced by crucifers (Louda and Mole, 1991), are repellent or even toxic to insects which do not feed on them. By contrast, most feeding stimulants are primary plant metabolites, and effective on a wide range of insects (Bernays and Chapman, 1994).

Dethier (1947) recognised that “no one attractant alone performs the service of guiding an insect to its proper host-plant, food or mate, and the desired end is achieved only by a complex array of stimuli, such as chemical, light, temperature and humidity, acting in harmony”. Thus, chemical attractants may be a blend of several compounds in a closely defined range of concentrations and ratios, often acting synergistically. Some, particularly primary metabolites (e.g. Hamamura, Hayashiya, Naito, Matsuura and Nishida, 1962), may be inactive on their own but have a synergistic effect on other compounds. Many are repellent at high concentrations (Dethier, 1947). For instance, neonate cabbage root fly larvae are attracted to allyl and ethyl isothiocyanate, but repelled by higher concentrations (Košťál, 1992).

3.1.2. The environment and behaviour of soil-dwelling larvae

In a review of environmental influences on soil insect behaviour, Villani and Wright (1990) raised a number of issues that are very important when designing or choosing bioassays to test insect responses to semiochemicals. Bioassays should simulate as far as possible the soil environment, while standardising or removing any stimuli other than those under study.

Soil is usually considerably wetter than surrounding air; relative humidity is usually within 2% of saturation. A number of other factors may affect relative humidity; soil moisture content, temperature and saturation pressure. It is very important in

bioassays that temperature should be similar to ambient temperature at the time of insect activity in the field, and should remain constant; sudden changes in temperature can increase insect activity.

It is difficult to separate water movement and vapour movement in the soil; there is a constant cycle of vapourisation, vapour flow, condensation, and short-range flow of liquids which then revapourise. Many smaller insects move in the thin films of water on soil particles, and are thus less likely to be impeded by the gross soil structure. It can readily be observed that neonate wheat bulb fly (WBF) larvae cannot move and rapidly desiccate in the absence of moisture. All bioassays therefore have to be moist, so it would be impossible to test only the diffusion of semiochemicals through air; it may, however, be possible to test whether they operate by diffusion through air and soil water, or by diffusion through soil water alone (Villani and Wright, 1990).

The metabolism of soil-dwelling organisms, especially plants, leads to higher levels of carbon dioxide in the soil than in the atmosphere. CO₂ diffuses far in soil air, but it is also very soluble in water, so any concentration gradient away from its source would be quite weak (Villani and Wright, 1990).

Many soil-dwelling insect larvae exhibit a thigmotactic response. Bernklau and Bjostad (1998) have observed this in the western corn rootworm (*Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)). WBF neonate larvae will continue to circle around the perimeter of a Petri dish once they have reached it. Although they need moisture to move, they are held in droplets of water by surface tension, and the water must be dispersed or they must be on a rough surface to move.

Many soil-dwelling insect larvae are photophobic, and this habit, too, can readily be observed in WBF neonate larvae. In order to simulate soil conditions, bioassays should be conducted in dark or near-dark conditions.

The larvae of WBF's close relative, the cabbage root fly, have a geotactic response (Finch and Thompson, 1992), but (Long, 1957) found that WBF larvae hatching from buried eggs moved upwards. However, it was unclear whether this behaviour was due to negative geotaxis, or whether it was in response to other stimuli.

3.1.3. Design of choice test bioassays for soil-dwelling larvae

Finch (1980) laid down some ground rules for bioassays testing the chemical attraction of phytophagous insects to plants:

- The quality and quantity of the stimulus must be controlled
- Other factors must be standardized
- Test insects must be isolated from plant material
- Test insects must be used only once, at their most responsive age
- The bioassay must select only one response
- The response of one individual may affect others
- There may be seasonal variations in response

Furthermore, the presence or absence of a choice of stimuli may affect insect response.

A number of different bioassays have been used in identifying attractants for soil-dwelling insect larvae. Most have tested stimuli as liquids, and only a few have tested gaseous stimuli (e.g. Jones and Coaker, 1977; Bernklau and Bjostad, 1998). In some cases (eg. Jones & Coaker, 1977; Schumann & Kühne, 1989; Košťál, 1992), the route of the larva towards the stimulus has been studied, as well as the attractancy of the stimulus itself.

Bernklau and Bjostad (1998) tested the responses of western corn rootworm larvae to maize (*Zea mays* L.) root volatiles and CO₂. They filled a vertically-mounted Y-

shaped glass tube of 9mm internal diameter with small glass beads and introduced first instar larvae into the middle upright arm. This exploited the larvae's geotropic and thigmotactic responses.

Such an apparatus is very attractive as a bioassay, since it simulates the soil in many respects, offers the larvae a very clear choice, and could be adapted to test liquid or gaseous stimuli. Furthermore, samples can be taken of stimulus and control air near the ends of the arms of the Y-tubes, analysed using GC-MS, and compared to samples taken from the root headspace of the host plant. The relationship between concentrations of stimulants in the bioassay, and concentrations of the same stimulants in the root headspace can thus be determined.

Jones and Coaker (1977) studied the reactions of all three instars of carrot fly (*Psila rosae* Fabricius) larvae. Their first instar larvae were placed on a 9cm petri dish of agar which had been lightly dusted with talcum powder in order to trace the route of the larvae.

Most other bioassays using first instar larvae (eg. Ross and Anderson, 1992, with carrot fly; Soni and Finch, 1979, with onion fly, *Delia antiqua* (Meigen)) have placed the larvae in the centre of a 9cm circle of moistened black filter paper with the stimulus and a control presented in opposite quarters of the circle.

Jones and Coaker (1977) tested the response of first instar carrot fly larvae to CO₂ by introducing the gas through a finely drawn-out glass pipette (internal diameter 0.025-0.05 mm) in the lid of a 9cm petri dish, attached to a manometer. This was in the centre of the petri dish lid, but the same method could be used to introduce CO₂ over the stimulus position adopted by Soni and Finch (1979) or Ross and Anderson (1992).

With all the above choice test bioassays, larvae were left in darkness or near-darkness for 15 or 30 minutes, after which the numbers of larvae in test, control, and neutral sections of the bioassay were recorded and analysed.

3.1.4. Previous work with wheat bulb fly larvae

Stokes (1956) first reported a chemotactic response in WBF larvae to wheat seedling exudates. In choice test bioassays, neonate larvae chose cubes of alginate gel in which wheat seedlings had been grown over control cubes of gel. Cut wheat shoots could be detected by the larvae when both were on damp filter paper, but this effect was reduced when a cover glass was laid under the shoots. This implied that the semiochemical(s) responsible were water-soluble exudates rather than volatiles.

From similar bioassays, and pot trials, Long (1958a) concluded that plant location by WBF larvae did not occur as the result of random movement, but involved a larval response to attractant material exuding from the plant. His results indicated that wheat stems played the major rôle in this process, and that attractive roots close to the base of the plant may actually interfere with infestation. His bioassays were conducted in the dark, and he found that the attractiveness of the exudates was reduced by boiling.

Scott (1974) repeated Stokes' (1956) results in choice test bioassays, but found that, if larvae were removed from either gel as soon as they reached it, there was no significant difference between the numbers of larvae arriving at wheat or control gels. He thus concluded that the larvae moved at random until they encountered a gel, and were held at wheat gels by arrestant exudates, rather than drawn to them by attractants. Although not explicitly stated, the methods used indicate that these bioassays were conducted in daylight, a fact later confirmed by Denholm (pers. comm.).

Using a series of single-choice "target arrestancy tests" developed by Scott (1974), Greenway, Scott, Calam and Smith (1976) concluded that the "arrestant" in wheat

extracts was probably a phenolic glycoside, and that oat extracts contained an “anti-arrestant” (a deterrent in Nordlund’s (1981) terms). These and all subsequent target arrestancy tests were also conducted in daylight (Denholm, pers. comm.).

Scott (1974) tested extracts of rye, barley, oats, and 5 grass species (but not couch) in the target arrestancy test. All except oats and *Festuca pratensis* Huds. gave similar “arrestancy values” to wheat. The arrestancy value for *F. pratensis* was significantly less than wheat, but significantly more than control gels. Oat extract had no arrestant effect, and when combined with wheat extract, the arrestant effect of wheat was reduced.

It is very rare to find more than one larva per wheat tiller, and even when this does occur, only one larva will survive (Long, 1960a). This suggests that there is some mechanism by which neonate larvae detect previously invaded tillers. It is not known whether this phenomenon occurs with other host plants.

Long (1958a) observed that larvae in the field preferred fresh plants to those already infested, and showed in laboratory experiments that this was “due to the [wheat] exudate being less attractive and not to the production of a repellent substance”. Scott and Greenway (1973) “found no differences in the arrestant properties of extracts from attacked and healthy wheat shoots in laboratory tests. Also, an extract of actively feeding second instar larvae dissected from attacked wheat plants did not arrest other larvae or produce an “anti-arrestant” effect when added to wheat.”

Despite all this research, there are still significant gaps in our understanding of host plant location by WBF larvae. The response of larvae to couch or its exudates and extracts has never been tested in laboratory bioassays, despite indications from pot trials that larvae prefer couch to wheat seedlings (Raw and Stokes, 1958). In direct contrast to Long (1958a), Scott (1974) concluded that wheat and its exudates and extracts had an arrestant, rather than an attractant, effect on WBF larvae. However, it would be remarkable if the larva could find its host entirely by random movement, in

view of the considerable distance it may have to travel (Long, 1958a). It can readily be observed that WBF larvae move away from light (negative phototaxis), but the effects of this reaction on bioassay results has rarely been considered. Several researchers (Gemmell, 1927; Long, 1957) have reported that WBF larvae move upwards in the soil after hatching (possibly due to negative geotaxis). However, larval geotaxis and phototaxis have never been experimentally tested and reported.

3.1.5. Aims of the current research

These bioassays were intended to establish whether neonate WBF larvae:

- choose wheat seedlings over couch seedlings or rhizomes
- choose wheat exudates over couch exudates
- exhibit positive or negative phototaxis or geotaxis
- are attracted or repelled by other neonate WBF larvae
- are repelled by damaged wheat seedlings or those already infested by WBF larvae

By visualising and analysing the trails left by neonate larvae, the present study aimed to establish whether couch, wheat or their exudates had a behavioural effect on WBF neonate larvae that was attractant, arrestant, or both.

Most bioassays of Anthomyiid larvae have used more than one larva in each replicate. This assumes that there is no interaction between larvae; an assumption which has never been experimentally tested or reported.

3.2. Materials and methods

3.2.1. Wheat bulb fly larvae

WBF larvae used in bioassays were reared from eggs collected from the field, as described in Chapter 2. In 1999, these larvae were subsequently reared to adulthood, but the adults lay too few eggs to be used for larval bioassays, so the process of collecting and storing eggs was repeated in autumn 1999.

WBF eggs stored as in Chapter 2 were surface sterilised in 1% NaOCl for 30 seconds, transferred to 9cm discs of moist black filter paper, and kept in a controlled climate cabinet at 10°C for 9 hours in weak light, 2°C in 15 hours darkness, and 80% RH., typical of local field conditions at the end of January. Each day, the filter paper was moistened if needed, and neonate larvae were removed for bioassays.

3.2.2. Interaction bioassay

Two NMR caps 1cm in diameter were glued to the inside of a plastic 9cm diameter Petri dish externally marked into quarters. Four small holes were made with a hot needle in the NMR caps to allow gaseous exchange with the rest of the Petri dish interior. In the inverted lid of the dish was a 9cm diameter circle of black filter paper moistened with 1ml distilled water. After 10 minutes, a neonate larva (stimulus) was placed under an NMR cap and the dish tightly secured with a rubber band. After a further 15 minutes, another (test) larva was placed in the centre of the filter paper, and the whole dish secured again (Fig. 3.1). After another 20 minutes, the position of each test larva was recorded as stimulus sector, control sector, or neutral sector.

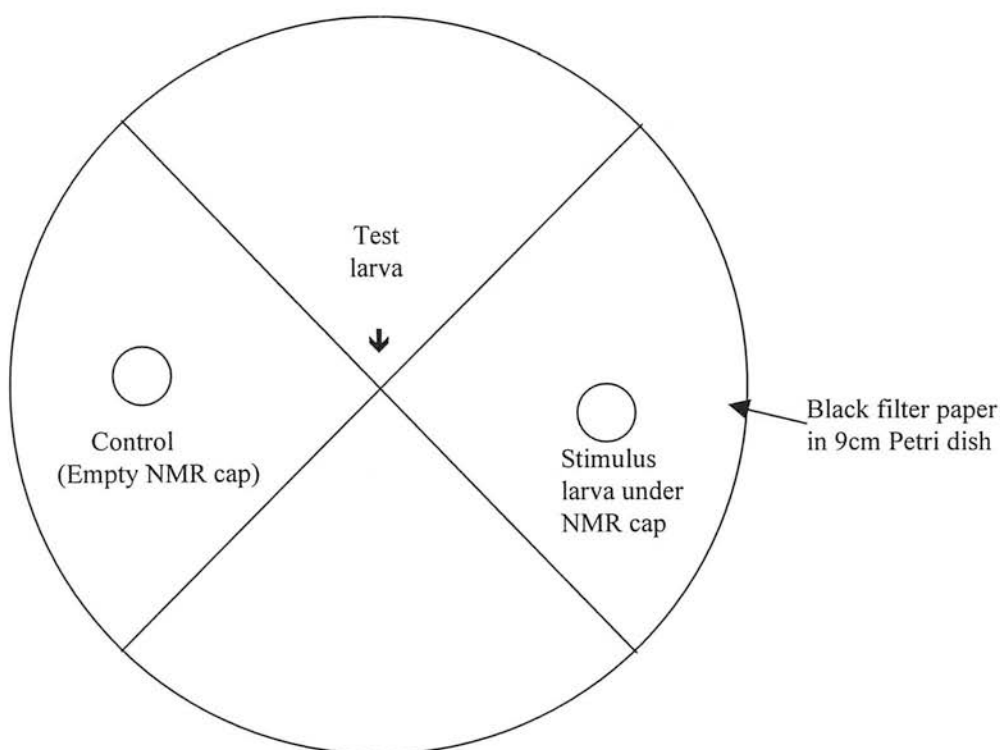


Fig. 3.1. Wheat bulb fly larval interaction bioassay

3.2.3. Geotaxis bioassay

Ten neonate larvae were transferred to a 1cm disc of black filter paper, and placed in the centre of a 9cm disc of black filter paper previously moistened with 0.8ml distilled water. This in turn lay in the centre of a 14cm diameter plastic Petri dish, which was divided into quarters by markings on the lid (Fig. 3.2). Ten such dishes were laid vertically on their side, and after 20 minutes the numbers of larvae in each quarter of the Petri dish (denoted up, down or neutral), and on the central 1cm disc, were recorded. Results were analysed with a χ^2 test of means of Arcsin-transformed data.

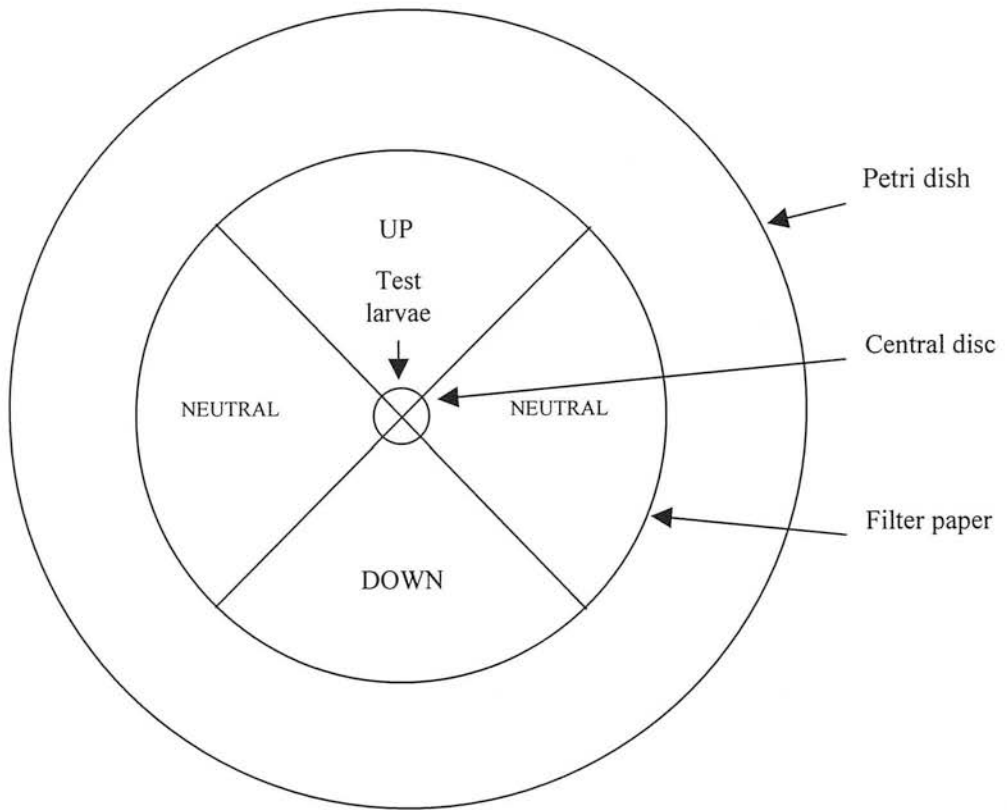


Fig. 3.2. WBF larval geotaxis bioassay

3.2.4 Phototaxis bioassay

A specially constructed plastic box was divided into ten compartments 2x2x10cm long. The lid and all the walls were of black plastic, with the exception of one end wall (2x2cm) of each compartment, which was of transparent plastic. In each compartment, a 2x9cm strip of black filter paper was moistened with 0.1ml distilled water. Fifteen minutes later, 10 neonate larvae were transferred to a 1cm diameter disc of black filter and placed in the centre of each compartment. The whole box was replaced in the darkened controlled climate cabinet where the larvae had hatched, and a neon strip light 59cm long, emitting a spectrum of wavelengths similar to daylight, was shone through the transparent side of the box (Fig. 3.3.). Twenty minutes later, the positions of the larvae were recorded.

Results were analysed with a χ^2 test of means of Arcsin-transformed data.

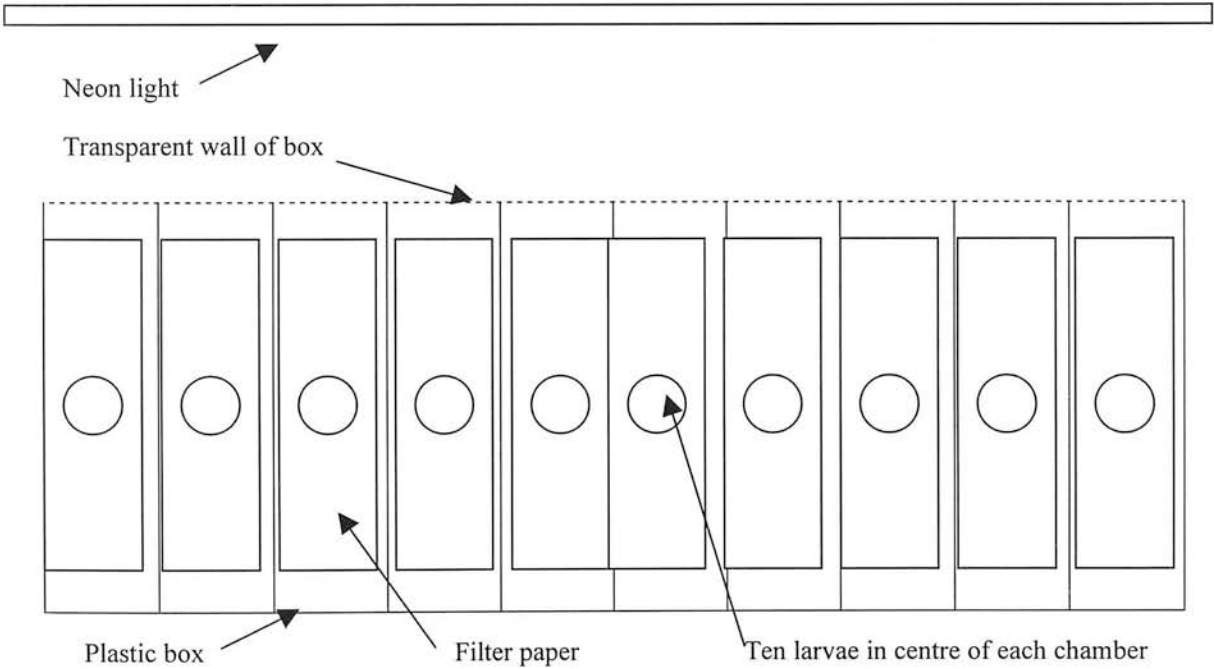


Fig. 3.3. WBF larval phototaxis bioassay (schematic plan, not to scale)

3.2.5. Host plant choice bioassays

A glass-bead bioassay, as described by Bernklau and Bjostad (1998), was tested with neonate cabbage root fly larvae and a stimulus of a cube of swede (*Brassica napus* L. var. *napobrassica*). No larval movement was detected, so this method was rejected.

Wheat seeds (cv Mercia, from SAC trial plots), untreated with fungicides or insecticides, were sterilised in 3% NaOCl for 2 minutes, thoroughly rinsed with sterile distilled water, then germinated on Whatman 181 paper moistened with sterile distilled water in the dark at 13°C. Untreated couch seeds (Herbiseed Ltd, Wokingham) were sterilised in the same way, but germinated at 10°C in the dark and 25°C in light, with a 12 hour day. Sections of couch rhizomes, length 1cm, which included a node and roots, were excised from plants originating from the material described in Chapter 2.1, and germinated under the same conditions as couch seeds.

Once coleoptiles were at least 2cm long (usually after 4 days), they were transplanted to alginate gel containing 1.5% sodium alginate and 0.3% calcium citrate by weight (after Scott, 1974). Seedlings for whole plant treatments were grown in inverted plastic cellular trays filled with alginate gel, with a shoot protruding through the hole in the bottom of each cell. Each cell was 16mm deep, and 10mm square at the top, tapering to 6mm square at the bottom. Blocks of 16 cells were placed in 1 litre glass beakers. Seedlings for exudate treatments were transplanted to a 16mm deep layer of gel in a 1 litre glass beaker.

Different treatments were kept in separate beakers sealed with Parafilm to avoid contamination between treatments with volatile compounds. The Parafilm was replaced daily to allow for gaseous exchange. The transplanted seedlings, and control treatments of plain gel, were kept under the same conditions as the hatching WBF larvae. After one week in these sealed containers, whole plants or gel plugs were removed for bioassays.

Pairs of whole plant treatments or exudate treatments were tested in a Petri dish bioassay very similar to that used for the interaction bioassays (Section 3.2.2), following the methods of Soni and Finch (1979). A 9cm diameter disc of black filter paper was placed in the centre of the inverted lid of a 14cm diameter plastic Petri dish. The filter paper was moistened with 1ml sterile distilled water, and the gel plugs being tested were placed 4cm apart, in the positions marked “Control” and “Stimulus” in Fig. 3.1. Whole plant bioassays were covered with an 18cm diameter x 5cm deep Perspex dish, while exudate bioassays were covered with the base of the 14cm plastic Petri dish. After at least one hour in a darkened controlled climate cabinet at 15°C, ten neonate larvae were transferred to the central 1cm of the filter paper disc, and the dish was returned to the cabinet. After a further twenty minutes, the numbers of larvae were recorded in each sector (control, stimulus or neutral), on each gel plug (control or stimulus), and remaining on the central disc.

The following comparisons were made:

- Wheat seedlings vs. Couch seedlings
- Wheat seedling exudate vs. Couch seedling exudate
- Wheat seedlings vs. Couch rhizomes
- Wheat seedling exudate vs. Couch rhizome exudate
- Wheat seedlings vs. Control
- Wheat seedling exudate vs. Control
- Couch seedlings vs. Control
- Couch seedling exudate vs. Control
- Couch rhizomes vs. Control
- Couch rhizome exudate vs. Control

Results were analysed with a χ^2 test of means of Arcsin-transformed data. Replicates where more than 50% of the larvae remained in the centre were not considered in this analysis. To save time and resources, in all but the final bioassay, fresh replicates were discontinued once a significant result had been achieved.

3.2.6. Analysis of trails left by larvae in host plant choice bioassays

Neonate WBF larvae leave hygroscopic trails on moist black filter paper that can be easily visualised by placing a small drop of water on their starting position. As soon as each attractancy bioassay was completed, a small drop of water was added to the centre of the filter paper, and the trails thus revealed were traced on a sheet of acetate film fixed to the outside of the dish above. Trails of all larvae coming within 1.9 cm of the centre of an odour source were then analysed using an adaptation of the methods described by Jones and Coaker (1977). A worked example is given in Appendix 2.

The area around each potential odour source, whether control or stimulus, was divided into two zones, with radius 2.4 cm (Zone 1) and 1.4 cm (Zone 2) (Figs. 3.4a and b).

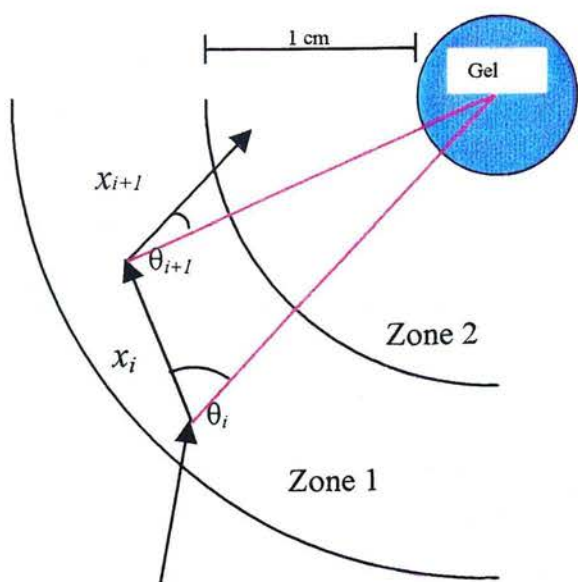


Fig. 3.4a. Calculation of attractancy parameter, S

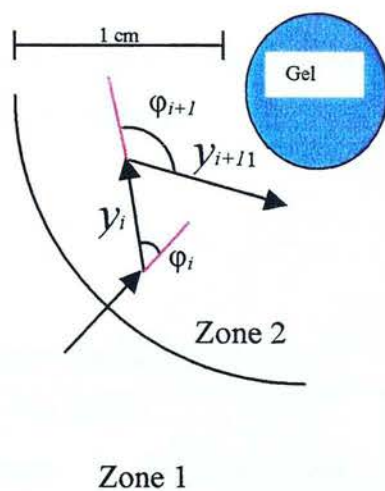


Fig. 3.4b. Calculation of arrestancy parameter, K

In Zone 1 the trail was divided into straight sections between each change in direction, and the length of each piece of straight trail (x mm) was measured together with the angle (θ) between it and a line from the start of the new direction to the

centre of the odour source (Fig 3.4a). $\sum x_i \theta_i$ was then calculated for all the observations in Zone 1. The parameter S , which gives an indication of the directness of the track towards the centre of the odour source, was calculated as:

$$S = \sum x_i \theta_i / 180 \sum x_i$$

When S has a value of 0, this represents a straight line towards the centre of the odour source, and when it has a value of 1, a straight line away from the source. Therefore the smaller the value of S , the stronger the suggestion of an oriented movement towards the odour source (i.e. attractancy), rather than a random approach.

In Zone 2, the track was similarly divided into straight sections, but here the total change in angle was recorded, i.e. the sum of the turned angles (ϕ) between successive straight sections (Fig. 3.4b). From this, the second parameter, K , was obtained as:

$$K = \sum \phi_i / \sum x_i$$

A high value of K therefore indicates a high turning tendency near the odour source (i.e. arrestancy), whereas a low value indicates a low turning frequency.

3.3 Results

3.3.1 Interaction bioassay

Table 3.1. Mean percentage of WBF larvae found in each part of interaction bioassay

Number of Replicates	Mean percentage of larvae in:		
	Neutral sectors	Control sector	Stimulus sector
9	66.66	22.22	11.11

In most cases, the test larvae did not move to either the control sector or the stimulus sector. The proper method of analysis would be a binomial distribution. However, from these partial results, it can be seen that a very large number of replicates would be needed to produce any significant results. The amount of time and resources required could not be justified, so, as in all previous research, the assumption that neonate larvae do not interact will have to be accepted.

3.3.2 Geotaxis bioassay

Table 3.2. Mean percentages of WBF larvae found in each part of geotaxis bioassay

Number of replicates	Mean percentages of larvae in or on:		
	Neutral disc and sectors	Upper sector	Lower sector
10	32.56	17.44	50

χ^2 tests on means of Arcsin-transformed data showed that a significantly higher percentage ($P<0.01$) of larvae moved down than moved up (Fig. 3.5). 95% confidence intervals are shown.

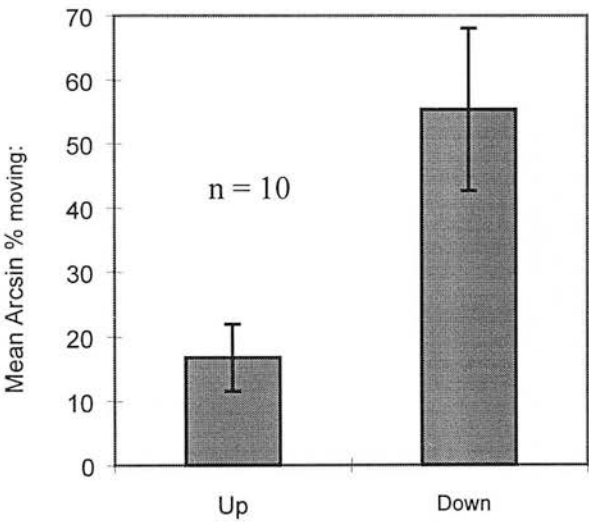


Fig. 3.5. WBF larval geotaxis

3.3.3. Phototaxis bioassay

Table 3.3. Mean percentages of WBF larvae found in each part of phototaxis bioassay

Number of replicates	Mean percentages of larvae in:		
	Neutral sector	Light sector	Dark sector
4	21.62	5.41	72.97

χ^2 tests on means of Arcsin-transformed data showed that a significantly higher percentage ($P<0.05$) of larvae moved into the dark than into the light (Fig. 3.6). 95% confidence intervals are shown.

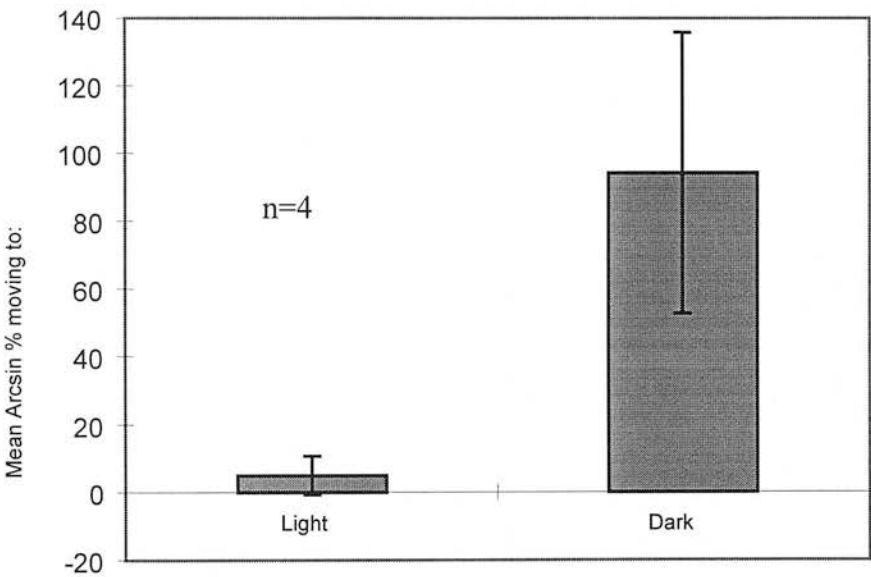


Fig. 3.6. WBF larval phototaxis

3.3.4. Host plant choice bioassays

Table 3.4: Mean percentages of WBF larvae in each part of host plant choice bioassays

No. of replicates	Mean percentages of larvae in or on:					
	Neutral		Wheat seedling		Couch seedling	
	centre	sectors	gel	sector	gel	sector
7	30.36	25	1.79	8.93	16.07	17.86
	Neutral		Wheat exudate		Couch seedling exudate	
	centre	sectors	gel	sector	gel	sector
14	28.68	33.33	3.88	9.30	5.43	19.38
	Neutral		Wheat seedlings		Couch rhizomes	
	centre	sectors	gel	sector	gel	sector
5	17.54	43.86	5.26	15.79	5.26	12.28
	Neutral		Wheat exudate		Couch rhizome exudate	
	centre	sectors	gel	sector	gel	sector
6	7.41	46.30	3.70	11.11	7.41	24.07
	Neutral		Control		Wheat seedlings	
	centre	sectors	gel	sector	gel	sector
4	37.5	20	0	12.5	12.5	17.5
	Neutral		Control		Wheat exudate	
	centre	sectors	gel	sector	gel	sector
10	27.73	30.04	0	16.18	12.71	17.33
	Neutral		Control		Couch seedlings	
	centre	sectors	gel	sector	gel	sector
4	43.24	18.92	5.41	5.41	13.51	13.51
	Neutral		Control		Couch seedling exudate	
	centre	sector	gel	sector	gel	sector
16	28.75	28.13	3.75	11.25	11.88	16.25
	Neutral		Control		Couch rhizome	
	centre	sector	gel	sector	gel	sector
2 *	45	30	0	20	0	5
	Neutral		Control		Couch rhizome exudate	
	centre	sector	gel	sector	gel	sector
5	28.57	30.61	0	6.12	4.08	30.61

* N.B. Data too few for reliable analysis, but included for completeness

Replicates where more than 50% of the larvae remained in the centre were rejected.

The data were Arcsin transformed and χ^2 tests were used to compare:

- percentages of larvae in each sector, excluding the neutral centre (active larvae)
- percentages of larvae outside the neutral sectors (responsive larvae) reaching a gel.

These figures are shown in Table 3.5. below:

Table 3.5. WBF larval response in host plant choice bioassays

No. of replicates	Mean Arcsin % active larvae reaching sector:			Mean Arcsin % responsive larvae reaching gel:		
	Couch seedling vs. wheat seedling					
	Wheat	Couch	See Fig.	Wheat	Couch	See Fig.
7	19.97	48.68 *	3.7a	3.61	37.77 *	3.7b
	Couch seedling exudate vs. wheat exudate					
	Wheat	Couch	See Fig.	Wheat	Couch	See Fig.
14	20.36	41.26 *	3.8a	7.58	17.92	3.8b
	Wheat seedlings vs. couch rhizomes					
	Wheat	Couch	See Fig.	Wheat	Couch	See Fig.
5	26.85	22.50	3.9a	8.05	8.23	3.9b
	Couch rhizome exudate vs. wheat exudate					
	Wheat	Couch	See Fig.	Wheat	Couch	See Fig.
6	14.43	34.18	3.10a	10.98	8.45	3.10b
	Wheat seedling vs. Control					
	Control	Wheat	See Fig.	Control	Wheat	See Fig.
4	24.16	47.41	3.11a	0	30.13 **	3.11b
	Wheat exudate vs. Control					
	Control	Wheat	See Fig.	Control	Wheat	See Fig.
10	20.88	43.06	3.12a	0	24.74 **	3.12b
	Couch Seedling vs. control					
	Control	Couch	See Fig.	Control	Couch	See Fig.
4	17.72	50.76 *	3.13a	12.63	40.61	3.13b
	Couch rhizomes vs. control					
	Control	Couch	See Fig.	Control	Couch	
2	40.55	8.37	3.14	0	0	
	Couch seedling exudate vs. Control					
	Control	Couch	See Fig.	Control	Couch	See Fig.
16	20.67	42.11 *	3.15a	7.99	24.28	3.15b
	Couch rhizome exudate vs. Control					
	Control	Couch	See Fig.	Control	Couch	See Fig.
5	9.08	49.70 *	3.16a	0	14.50	3.16b

* denotes a result significantly greater ($P < 0.05$) than the relevant comparison.

** denotes a result significantly greater ($P < 0.01$) than the relevant comparison.

Figures 3.7a to 3.16b show these results.

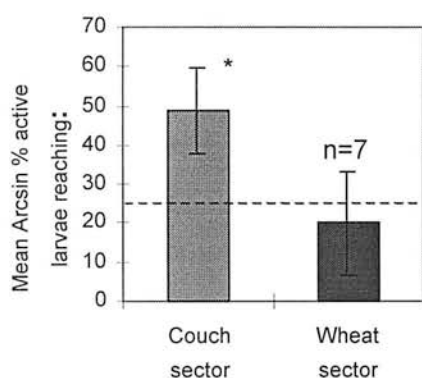
95% confidence intervals are shown by bars.

The broken horizontal lines indicate 25%, the percentage of active larvae which might be expected to reach either sector by chance.

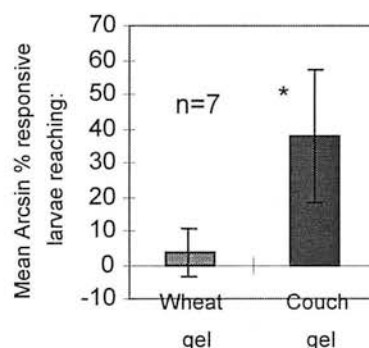
* denotes a result significantly greater ($P < 0.05$) than the relevant comparison.

** denotes a result significantly greater ($P < 0.01$) than the relevant comparison.

Fig. 3.7. WBF larval response to couch vs. wheat seedlings

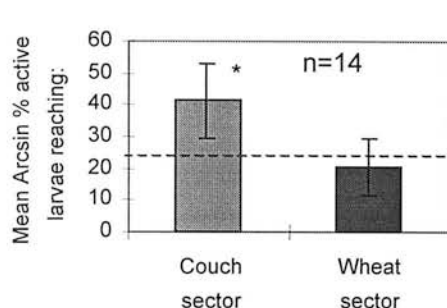
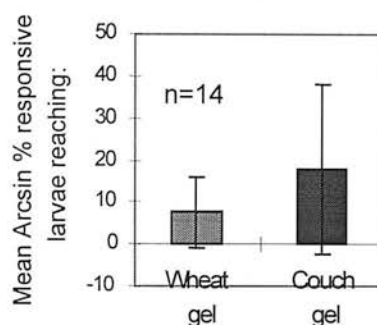


3.7a. Active larvae reaching wheat or couch sectors

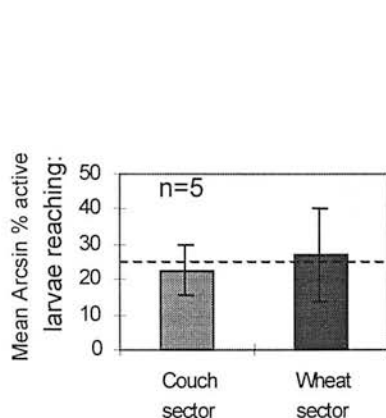
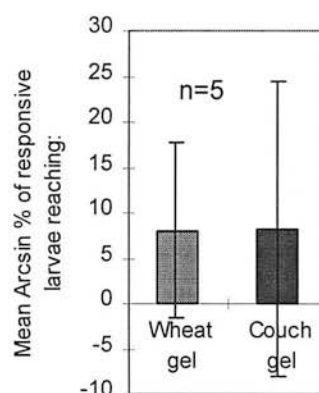


3.7b. Responsive larvae reaching wheat or couch gels

A significantly higher percentage of active larvae ($P < 0.05$) reached the couch seedling sector than reached the wheat seedling sector. A significantly higher percentage of active larvae ($P < 0.05$) reached the couch seedling sector than might be expected to reach that sector by random movement. A significantly higher percentage of responsive larvae ($P < 0.05$) reached the couch seedling gel than reached the wheat seedling gel.

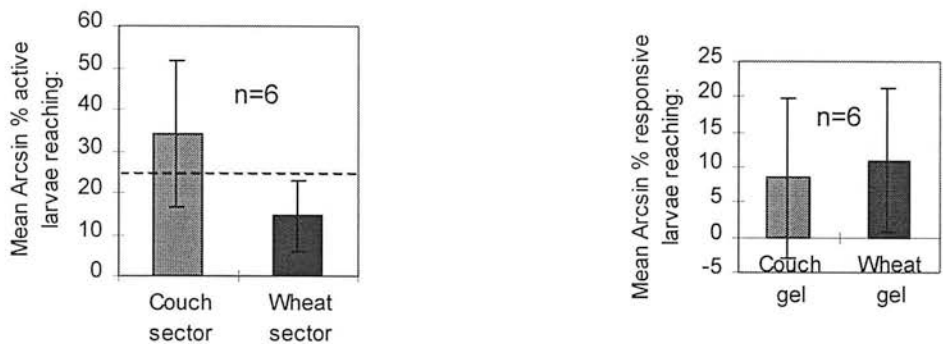
Fig. 3.8. WBF larval response to couch vs. wheat seedling exudates**3.8a.** Active larvae reaching wheat or couch sectors**3.8b.** Responsive larvae reaching wheat or couch gels

A significantly higher percentage of active larvae ($P < 0.05$) reached the couch seedling exudate sector than reached the wheat seedling exudate sector. A significantly higher percentage of active larvae ($P < 0.05$) reached the couch seedling exudate sector than might be expected to reach that sector by random movement.

Fig. 3.9. WBF larval response to couch rhizomes vs. wheat seedlings**3.9a.** Active larvae reaching wheat or couch sectors**3.9b.** Responsive larvae reaching wheat or couch gels

There was no significant difference between larval responses to couch rhizomes and to wheat seedlings.

Fig. 3.10. WBF larval response to couch rhizome vs. wheat seedling exudates

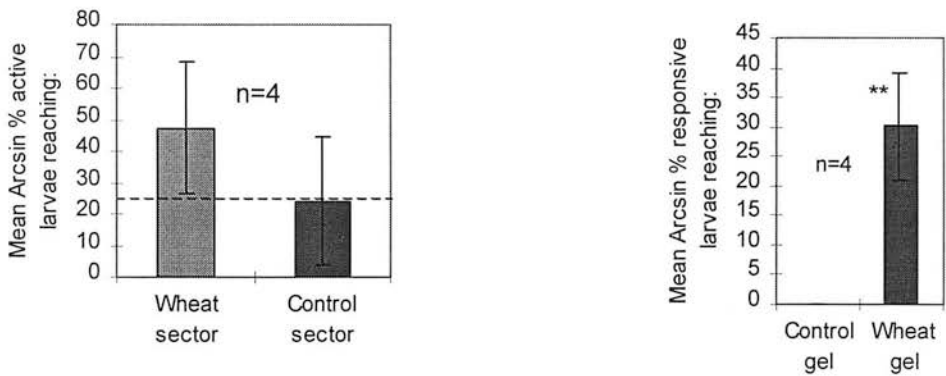


3.10a. Active larvae reaching wheat or couch sectors

3.10b. Responsive larvae reaching wheat or couch gels

A significantly lower percentage of active larvae ($P<0.05$) reached the wheat seedling sector than might be expected to reach that sector by random movement.

Fig. 3.11. WBF larval response to wheat seedlings vs. control

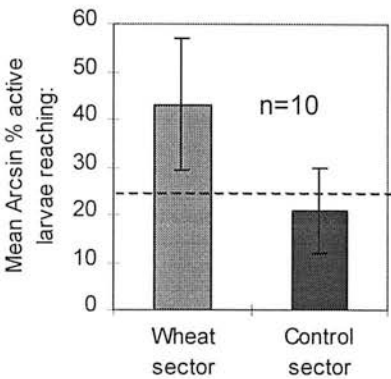


3.11a. Active larvae reaching wheat or control sectors

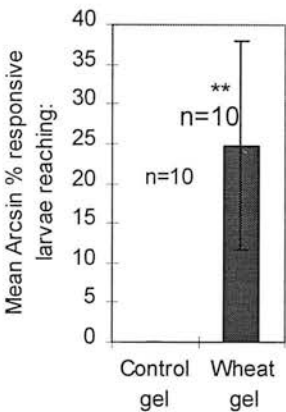
3.11b. Responsive larvae reaching wheat or control gels

A significantly higher percentage of active larvae ($P<0.05$) reached the wheat seedling sector than might have been expected to reach that sector by random movement. A significantly higher percentage of responsive larvae ($P<0.01$) reached the wheat seedling gel than reached the control gel.

Fig. 3.12. WBF larval response to wheat seedling exudate vs. control



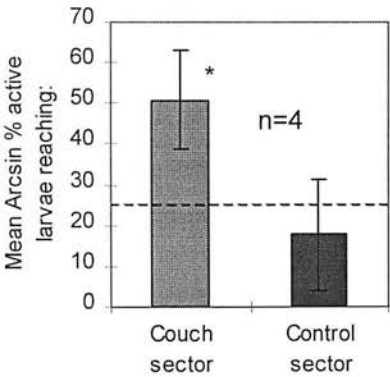
3.12a. Active larvae reaching wheat or control sectors



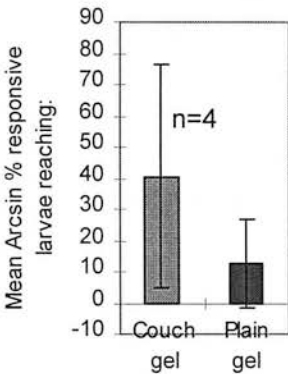
3.12b. Responsive larvae reaching wheat or control gels

A higher percentage of active larvae reached the wheat exudate sector than reached the control sector, but this difference was not quite significant. A significantly higher percentage of active larvae ($P<0.05$) reached the wheat exudate sector than might have been expected from random movement. A significantly higher percentage of responsive larvae ($P<0.01$) reached the wheat exudate gel than reached the control gel.

Fig. 3.13. WBF larval response to couch seedlings vs. control



3.13a. Active larvae reaching couch or control sectors

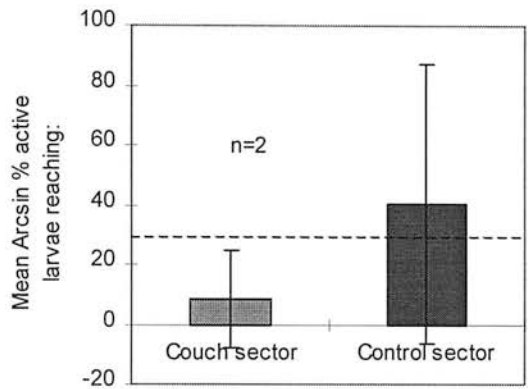


3.13b. Responsive larvae reaching couch or control gels

A significantly higher percentage of active larvae ($P<0.05$) reached the couch seedling sector than reached the control sector. A significantly higher percentage of

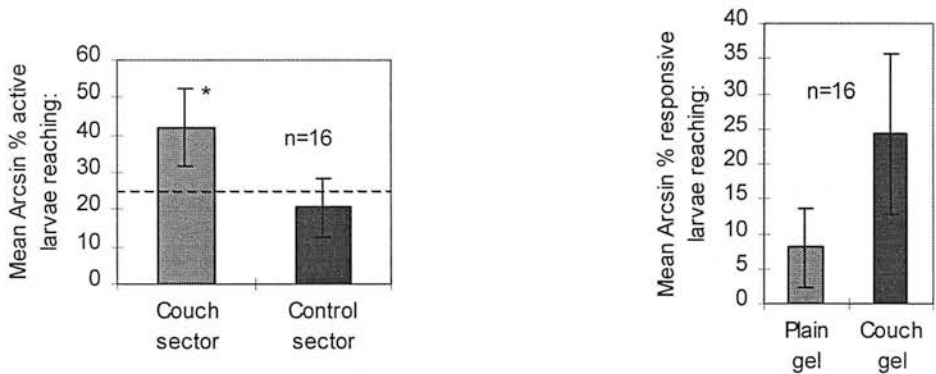
active larvae ($P<0.05$) reached the couch seedling sector than might have been expected to reach that sector by random movement.

Fig. 3.14. WBF larval response to couch rhizomes vs. control



There was no significant difference between the response of larvae to couch rhizomes and to control gels. However, these results are based on only two replicates.

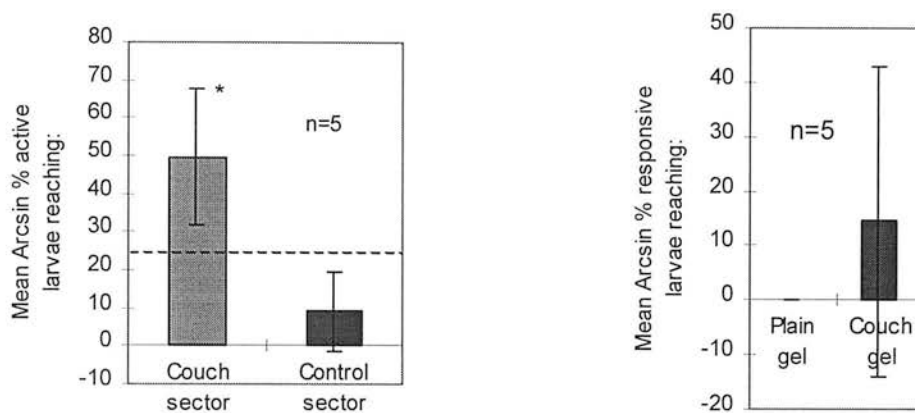
Fig. 3.15. WBF larval response to couch seedling exudate vs. control



3.15a. Active larvae reaching couch or control sectors

3.15b. Responsive larvae reaching couch or control gels

A significantly higher percentage of active larvae ($P<0.05$) reached the couch seedling exudate sector than reached the control sector. A significantly higher percentage of active larvae ($P<0.05$) reached the couch seedling exudate sector than might have been expected to reach that sector by random movement. A higher percentage of responsive larvae reached the couch seedling exudate gel than reached the control gel, although this difference was not quite significant.

Fig. 3.16. WBF larval response to couch rhizome exudate vs. control**3.16a.** Active larvae reaching couch or control sectors**3.16b.** Responsive larvae reaching couch or control gels

A significantly higher percentage of active larvae ($P < 0.05$) reached the couch rhizome exudate sector than reached the control sector. A significantly higher percentage of active larvae ($P < 0.05$) reached the couch seedling exudate sector than might have been expected to reach that sector by random movement. A significantly lower percentage of active larvae ($P < 0.05$) reached the control sector than might have been expected to reach that sector by random movement. No larvae reached the control gel, but the percentage of responsive larvae reaching the couch rhizome exudate was very variable, and hence not significantly higher.

3.3.5 Analysis of larval trails in host plant choice bioassays

Trails left by larvae in whole plant bioassays were not statistically analysed, as their numbers were too few, and the resulting S and K values were highly variable. It also proved difficult to accurately trace the trails onto an acetate sheet 7.5 cm above the filter paper.

S and K values for wheat and couch seedling exudates, and for control gels, showed a negatively skewed distribution, and were analysed using Mann-Whitney's non-parametric test.

3.3.5.1. S values (attractancy) of seedling exudates, and of control gels

Median S values in each bioassay are compared in Table 3.6, below

Table 3.6 Median S values for seedling exudates and controls
(lower S value = greater attractancy)

1. Couch seedling exudate vs. control				
Couch	n	Control	n	Significance
0.15	32	0.34	32	P<0.01
2. Wheat exudate vs. control				
Wheat	n	Control	n	Significance
0.19	24	0.35	11	NS
3. Couch seedling exudate vs. wheat exudate				
Couch	n	Wheat	n	Significance
0.33	21	0.29	14	NS
4. Wheat exudate vs. all control gels				
Wheat	n	Control	n	Significance
0.19	24	0.35	43	P<0.05
5. Couch exudate from different bioassays				
vs. Wheat	n	vs. Control	n	Significance
0.33	21	0.15	32	P<0.05
6. Wheat exudate from different bioassays				
vs. Couch	n	vs. Control	n	Significance
0.30	14	0.19	24	NS

Couch seedling exudate is significantly more attractive than the control gels ($P<0.01$). However, the lack of any significant difference in S between wheat exudate and control gels may be due to the small number of larvae attracted to control gels. When data for all control gels are pooled, wheat exudate is significantly more attractive ($P<0.05$) than control gels. Couch exudate is significantly more attractive ($P<0.05$) when compared with control gels than when compared with wheat exudate. This suggests that the attractancy of couch exudate is confounded in the presence of another attractant, such as wheat exudate. However, wheat exudate is no more attractive when compared with control gels than when compared with couch exudate.

These results would also suggest that S values of control gels are not significantly different from 0.5, a neutral value, that is, they are not significantly attractive.

3.3.5.2. K values (arrestancy) for seedling exudates and control gels

Direct comparisons within bioassays gave no significant results, but since there was also no significant difference between identical treatments in different bioassays, the results were pooled, and are presented in Table 3.7, below:

Table 3.7. Median K values for seedling exudates and controls
(Higher K value = greater attractancy)

1. Couch seedling exudate vs. control				
Couch	n	Control	n	Significance
9.90	40	10.83	23	NS
2. Wheat exudate vs. control				
Wheat	n	Control	n	Significance
13.51	24	10.83	23	P<0.01
3. Couch seedling exudate vs. wheat exudate				
Couch	n	Wheat	n	Significance
9.90	40	13.51	24	P<0.01

Wheat exudate is significantly more arrestant ($P<0.01$) than couch seedling exudate or control gels. Couch seedling exudate is not significantly more arrestant than control gels, but since a difference of 2.68 between wheat exudate and control K values is significant, one can assume that both couch seedling exudate and control gels have a K value that is significantly greater than 0, that is, they both have an arrestant effect.

3.4. Summary of results

WBF neonate larvae:

- are positively geotactic
- are negatively phototactic
- choose couch seedlings and their exudates over alginate gel controls
- choose couch rhizome exudates over alginate gel controls
- choose couch seedlings and their exudates over wheat seedlings and their exudates

Once within 2cm of a couch seedling, they are more likely to reach this than a wheat seedling. Once within 2cm of a wheat seedling or its exudate, they are more likely to reach this than alginate gel controls.

A higher percentage of larvae come within 2cm of couch seedlings, their exudates, or couch rhizome exudates than would be expected to do so by random movement. The same applies to wheat seedlings or their exudates only when compared to alginate gel controls. This suggests an attractant effect as well as, or perhaps even instead of, an arrestant effect.

Both couch and wheat seedling exudates are more attractive than all alginate gel controls aggregated together. However, when directly compared to control gels, only couch seedling exudate is more attractive. When couch and wheat seedling exudates are directly compared, the couch exudate is less attractive than when compared to controls.

Wheat exudate is more arrestant than couch seedling exudate or controls. However, control gels themselves may have some arrestant effect.

3.5. Discussion

The strong positive geotaxis demonstrated by neonate WBF larvae in the present study suggests that the upward movements in soil described by Long (1958a) can be attributed to the attractancy of wheat, rather than to any innate negative geotaxis.

Scott (1974), and Greenway *et al.* (1976) concluded that neonate WBF larvae find wheat exudates and extracts by random movement followed by an arrestant effect of the exudates and extracts. However, their bioassays were conducted in daylight. The present bioassays show that the larvae are strongly photophobic, and that, in the dark, wheat seedlings and their exudates have an attractant as well as an arrestant effect. Wheat exudates are, however, stronger arrestants than couch seedling exudates. The suggestion that control gels in these bioassays had some arrestant effect on WBF larvae needs further investigation; this would best be done with single choice bioassays, as used by Jones and Coaker (1977), comparing plain alginate gels with water controls.

One may thus hypothesise a catenary process in host location by neonate WBF larvae. Larvae hatching on the surface of the soil would first show a photophobic and geotactic response, thus avoiding dessication or predation. Then they would be drawn to the host plant stem by attractant(s), and kept in close proximity to the stem by arrestant(s). The present results show that couch exudates are more attractive than wheat exudates, and that wheat exudates are more arrestant than couch exudates; this suggests that at least two chemicals, one attractant and one arrestant, are involved.

It has often been suggested that couch grass is the natural host of WBF (Gemmell, 1927; McAlpine & Slight, 1981; Griffiths, 1992). In choice-test pot trials comparing infestation rates in wheat and other known host plants (Raw & Stokes, 1958), only couch plants had a higher infestation rate than wheat (133% relative to wheat). However, these trials were small-scale, and were not statistically analysed. The present study showed that neonate WBF larvae significantly chose couch seedlings and their exudates over wheat seedlings and their exudates. They also chose couch

rhizome exudates over controls. The strong response to couch rhizome exudates would merit further investigation, since, according to Holm *et al.* (1977), couch seedlings do not usually germinate until April or May, and are weaker and slower to develop than the aerial shoots of couch rhizomes. Neonate WBF larvae are thus more likely to encounter aerial shoots of couch rhizomes than couch seedlings.

These findings support the hypothesis that couch grass is the natural host of wheat bulb fly.

In the present study, time and resources did not permit an analysis of the chemistry of couch or wheat exudates. However, other studies have identified possible candidates for WBF attractants and arrestants in wheat and couch. Greenway *et al.* (1976) ascribed only arrestant properties to wheat extracts and exudates tested in bioassays of neonate WBF larvae, and concluded that the arrestant(s) were probably phenolic glycosides.

The hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), and its demethoxylated analogue DIBOA, are widespread in the Triticeae (Copaja, Barria & Niemeyer, 1991) and some wild Aveneae, but absent in oats (FuentesContreras, Powell, Wadhams, Pickett & Niemeyer, 1996; Gianoli & Niemeyer, 1998). Levels of both compounds vary widely in different parts of the plant (Copaja, Nicol & Wratten, 1999), in different species of the tribe Triticeae (Copaja *et al.* 1991; Niemeyer, Copaja & Barria, 1992), and indeed in different cultivars of wheat (Nicol, Copaja, Wratten & Niemeyer, 1992). Furthermore, different studies have used different methods of extraction and analysis, so direct comparisons of hydroxamic acid levels in different species are not always possible.

However, it is now clearly established that DIMBOA is exuded by the roots of both couch (Friebe, Schulz, Kück and Schnabl, 1995) and wheat (Wu, Haig, Pratley,

Lemerle & An, 2000), and that DIBOA is the precursor of DIMBOA (Frey, Chomet, Glawischnig, Stettner, Grün, Winklmaier, Eisenreich, Bacher, Meeley, Briggs, Simcox & Gierl, 1997). Niemeyer *et al.* (1992) found high levels of DIBOA but no DIMBOA in cultivated rye, and none of either chemical in cultivated barley. Perennial species of *Hordeum*, however, have moderate levels of DIBOA but no DIMBOA (Copaja *et al.*, 1991). Thus WBF's two most favoured host plants, couch and wheat, have higher levels of DIMBOA than less favoured or rejected species.

Copaja *et al.* (1999) found that hydroxamic acids were absent in wheat seeds, but that the concentration of DIMBOA in wheat seedling roots peaked 2-4 days after germination. The absolute amount of DIMBOA in the whole seedling, however, increased rapidly in the first four days after germination, and thereafter remained fairly constant until the study ended after 7 days, indicating a growth dilution effect. This suggests that the concentration of DIMBOA exuded into the soil around the plant would also remain fairly constant from day 4 to day 7, and possibly for longer.

Hydroxamic acids have deleterious effects on fungi, bacteria and insects attacking cereals (Niemeyer, 1988). It has been suggested that they also have an allelopathic effect on germinating species of other plants (Friebe *et al.*, 1995, Wu *et al.*, 2000). In wheat, they deter aphids (Nicol *et al.*, 1992) and reduce their performance (Niemeyer *et al.*, 1992). Iwamura, Nagakawa & Hirai (1996) found DIBOA and DIMBOA concentrations were highest in the root tip and the sub-epidermal tissues of the leaf sheath in young wheat seedlings, suggesting that these chemicals act as defence compounds in tissues exposed to insect and microbial attacks. If attack by WBF larvae reduces the output of larval attractants by wheat meristem, this might account for the finding that WBF larvae find previously infested shoots less attractive than fresh shoots (Long, 1960a).

There is no direct evidence of a behavioural response by WBF larvae to hydroxamic acids, but their rôle in host plant location by WBF clearly merits further investigation. However, constraints on time and resources did not allow for such studies in the current project. Attraction to DIMBOA, a compound with deterrent and toxic effects on other organisms, would be consistent with similar effects, such as the attraction of larvae of the closely-related cabbage root fly to glucosinolates (Košťál, 1992).

Chapter 4

Interaction with host plants during wheat bulb fly larval development

Chapter 4. Interaction with host plants during larval development

4.1. Introduction

Several studies have investigated wheat bulb fly (WBF) larval mortality in the field (Raw, 1967; Ryan, 1973b) and in laboratory cultures (Bardner and Kenten, 1957; Jones and Moore, 1978). Ryan (1973b) found larval mortalities after host plant invasion of 67-81%, and concluded that this was due to young larvae killing wheat plants and leaving insufficient food for older larvae, especially in the third instar. Even in optimal laboratory rearing conditions, Jones and Moore (1978) found that only 55-70% of neonate larvae became adults. Above 18°C, larval growth in the laboratory was so rapid that competition between second and third instar larvae for host plants caused a high mortality and few flies emerged (Jones & Moore, 1978).

Ryan (1973b) stated that WBF larvae need a total of 5 shoots to complete their development; one for the first and second instars, and the other four for the third instar. However, Jones (1970b, 1978) found that, even under optimal laboratory conditions, a maximum of 2.7 shoots per larva would suffice.

There have been conflicting reports on the ability of individual wheat plants and of wheat crops to compensate for attack by WBF. Griffiths and Scott (1969) found that single wheat shoots at the one-leaf stage, before any lateral buds had formed, were unable to survive artificial or WBF larval damage to the central meristem. Older plants with at least three leaves, however, were able to survive attack, because at this stage, according to Griffiths and Scott (1969), the meristem of the second shoot is well-developed and separate from the meristem of the first shoot which is destroyed first by the larva. In field experiments, Bardner, Fletcher and Huston (1969) found that attacked wheat plants were slow to grow, and survivors produced fewer shoots and ears than unattacked plants. Unattacked plants, however, were able to compensate for the reduced growth of their attacked neighbours. Long and Morris (1961) found that WBF damage retarded plant growth by up to 4 weeks and, although they subsequently produced more buds and shoots than undamaged plants, damaged plants failed to replace all the damaged shoots and fewer shoots survived to harvest.

It should be noted that all the above studies were undertaken on wheat, and none of WBF's other host plants has been similarly studied. A pot trial was designed to compare the development and mortality of wheat bulb fly larvae, and the mortality or recovery of their host plants, on wheat seedlings and couch shoots grown from rhizomes.

4.2. Materials and methods

Wheat seedlings (*Triticum aestivum*), cv. Mercia, and couch rhizomes (*Elytrigia repens*) were prepared as for the WBF larval bioassays (Chapter 3), and transplanted at a depth of 2cm into 20 cm diameter plastic pots full of peat (Scotts, Newbridge, Co. Kildare, Ireland) covered by a 2cm layer of horticultural sand (William Sinclair Horticulture Ltd, Lincoln). Each pot contained 2 plants in the centre, surrounded by 2 concentric circles of 8 and 10 plants, evenly spaced. One neonate larva, raised as described in Chapter 2, was placed beside each of the central 10 plants (Fig. 4.1).

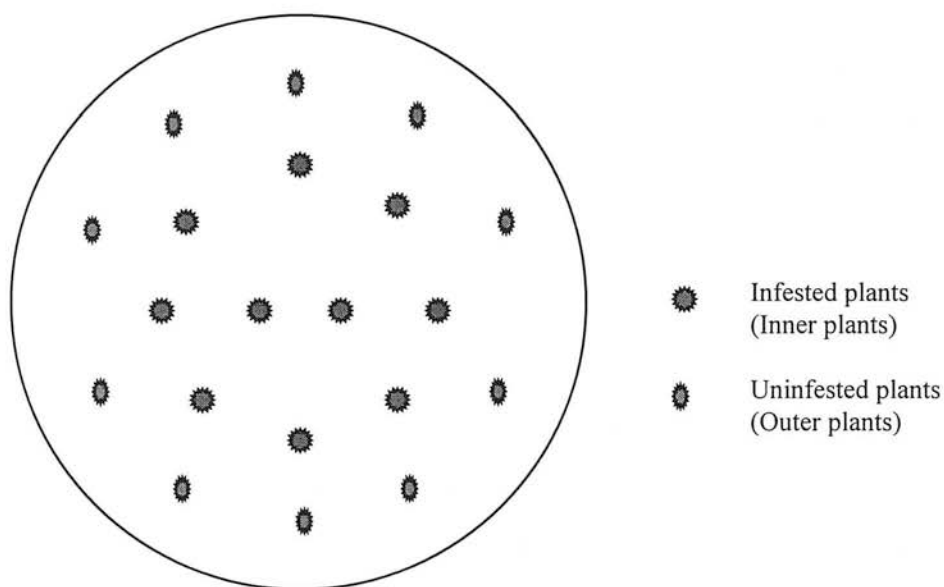


Fig. 4.1. Layout of plants in WBF larval / host plant interaction pot trial

Thirteen pots of each treatment were prepared in this way, although due to attack by mildew, not all were considered for analysis of results. Pots of infested plants, and identical pots of uninfested control plants, were kept at $13 \pm 2^\circ\text{C}$, 80% RH, 10 hours daylight for 8 weeks, and watered at the base twice weekly. Once a week, the numbers of leaves, shoots and dead-hearts were recorded on each plant, and dead-hearts were marked with a cocktail stick. For logistical reasons, it was not possible to start all

replicates at once; the first plants were transplanted on 27 January 1999, and the last on 1 June 1999.

After 8 weeks, each infested pot was transferred to a glasshouse at ambient temperature, and covered with a perspex tube 50cm high and a stocking to allow access to the pot. These pots were checked daily, and the sex and number of any adult WBF emerging were recorded. Newly-emerged adults were transferred to adult rearing cages. After all adults had emerged, the root and soil contents of each pot were washed and sieved to retrieve any uneclosed WBF pupae.

Newly-emerged adult WBF reared on couch and wheat were kept in separate cages in the glasshouse under similar conditions to those used by Jones and Moore (1978), with 4 couch and 4 wheat plants in each cage. The cages were 50 x 50 x 96cm high. Food was presented on dental wicks in 4 separate 5cm Petri dishes, placed on a solid perspex shelf resting on top of the plant pots, and replaced twice a week. The Petri dishes contained, respectively: sterile distilled water; 5ml citrated sheeps' blood diluted with 5ml water; 1g honey diluted with 9ml water; 1g each dried yeast and dried milk powder, diluted with 8ml water. Gravid females were transferred to separate oviposition chambers, as used by Bardner and Kenten (1957), and the numbers of eggs laid by couch-reared and wheat-reared flies were counted daily.

4.3 Results

Data were collated in Excel 5.0. and analysed using analysis of variance in Genstat 5.0. Results from pots where the plants had been severely affected by mildew were not analysed; after these had been discarded, six replicates of each treatment remained for analysis. Inner and outer rings of plants in each pot were considered as split plots for all treatments.

Furthermore, in pots which had been infested with WBF larvae, plants showing obvious signs of attack (Infested), and those showing no such signs (Clean) were considered as split plots. This measured the effect of WBF attack on individual plants rather than small populations (pots) of plants. In cases where there were no signs of attack on any

plants (all wheat replicates in week one, and wheat replicates 1 and 2 in week two), the figures for the inner ring of infested wheat plants were used as proxies for infested plants, for the purpose of analysis.

Means for each treatment were compared for each week. Data were also summarised across time by calculating the mean, linear and quadratic parameters of linear regressions for each treatment. These parameters were then compared by analysis of variance; they are only presented in the following tables where there are significant differences between treatments. The linear parameter measures the slope of the linear regression curve, i.e. the rate of change in the data. The quadratic parameter indicates the shape of the curve; a straight line gives a quadratic parameter of 0, while a concave curve (i.e. an increasing rate of change) gives a positive quadratic value, and a convex curve (i.e. a decreasing rate of change) gives a negative quadratic value.

Key to notation used in Figs. 4.2 to 4.12, and Tables 4.1 to 4.8

Treatments:	Differences between treatments:	
C = Couch	NS = No significant difference	
W = Wheat	* = Significant difference ($P<0.05$)	} to all other treatments
Ct = Control	** = Significant difference ($P<0.01$)	
If = Infested	*** = Significant difference ($P<0.001$)	
I = Inner	Different letters following means indicate a significant	
O = Outer	difference at the level of probability shown.	

4.3.1. Effect of WBF larvae on total number of shoots per plant

Considering the inner and outer rings of plants in each pot as sub-plots produced the following results:

Table 4.1. Effect of WBF larvae on mean number of shoots per plant

Treatment	Weeks after infestation								Regression parameters		
	1	2	3	4	5	6	7	8	Mean	Lin	Quad
C If I	1.23	1.63 *	2.18 *	2.38	2.68	2.88	3.08	3.33			
C If O	1.05	1.13	1.25	1.47	1.75	2.05	2.37	2.58			
W If I	1.00	1.00	1.12	1.37	1.72	2.20	2.70	3.07		No	
W If O	1.00	1.00	1.10	1.30	1.72	2.10	2.62	3.00		Significant	
C Ct I	1.00	1.03	1.03	1.38	2.03	2.75	3.12	3.43		Differences	
C Ct O	1.00	1.00	1.00	1.18	1.60	2.12	2.47	2.88			
W Ct I	1.00	1.00	1.00	1.02	1.37	1.70	2.02	2.35			
W Ct O	1.00	1.00	1.00	1.05	1.22	1.53	1.67	1.90			
Means of host plant effects:											
Couch	1.07	1.20 *	1.37 *	1.60 *	2.02	2.45 *	2.76	3.06	1.94 *	No Significant	
Wheat	1.00	1.00	1.05	1.18	1.50	1.88	2.25	2.58	1.55	Differences	
Means of treatment effects:											
Infested	1.07	1.19 *	1.41**	1.63 *	1.97 *	2.31	2.69	3.00	1.91 *	NSD	0.019 *
Control	1.00	1.01	1.01	1.16	1.55	2.03	2.32	2.64	1.59		0.037
Means of plant ring effects:											
Inner	1.06	1.17 *	1.33**	1.54**	1.95**	2.38***	2.73**	3.05**	1.9**	0.3 *	0.024
Outer	1.01	1.03	1.09	1.25	1.57	1.95	2.28	2.59	1.59	0.24	0.032 *
Host plant / treatment interactions:											
Couch Inf	1.14 *	1.38**	1.72**	1.93 *	2.22	2.47	2.73	2.96		0.26 b	-0.0027c
Wheat Inf	1.00	1.00	1.11	1.33	1.72	2.15	2.66	3.03	NSD	0.31ab	0.0406ab
Couch Cntrl	1.00	1.02	1.02	1.28	1.82	2.43	2.79	3.16		0.34 a	0.0421 a
Wheat Cntrl	1.00	1.00	1.00	1.03	1.29	1.62 *	1.84**	2.13		0.17 c	0.0312 b
								P		*	**
Host plant / plant ring interactions:											
Couch Inner	1.12	1.33 *	1.61**	1.88**	2.36**	2.82 *	3.10	3.38	2.2 a		0.0086 *
Couch Outer	1.03	1.07	1.13	1.33	1.68	2.08	2.42	2.73	1.68 b	NSD	0.0308
Wheat Inner	1.00	1.00	1.06	1.19	1.54	1.95	2.36	2.71	1.6 bc		0.0394
Wheat Outer	1.00	1.00	1.05	1.18	1.47	1.82	2.14	2.45	1.51 c		0.0324
								P	**		
Treatment / plant ring interactions:											
Inf Inner	1.12	1.32 *	1.65**	1.88**	2.20	2.54	2.89	3.20		No	
Inf Outer	1.03	1.07	1.18	1.38	1.73	2.08	2.49	2.79		Significant	
Cntrl Inner	1.00	1.02	1.02	1.20	1.70	2.23	2.57	2.89		Differences	
Cntrl Outer	1.00	1.00	1.00	1.12	1.41	1.83	2.07	2.39			

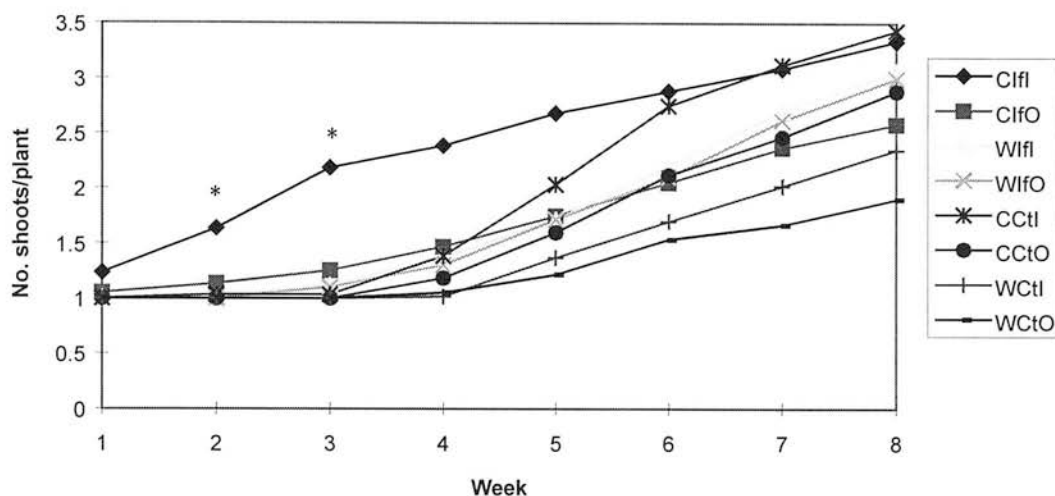


Fig. 4.2a. Effect of WBF larvae on mean number of shoots per plant (Inner and outer rings of plants as split plots)

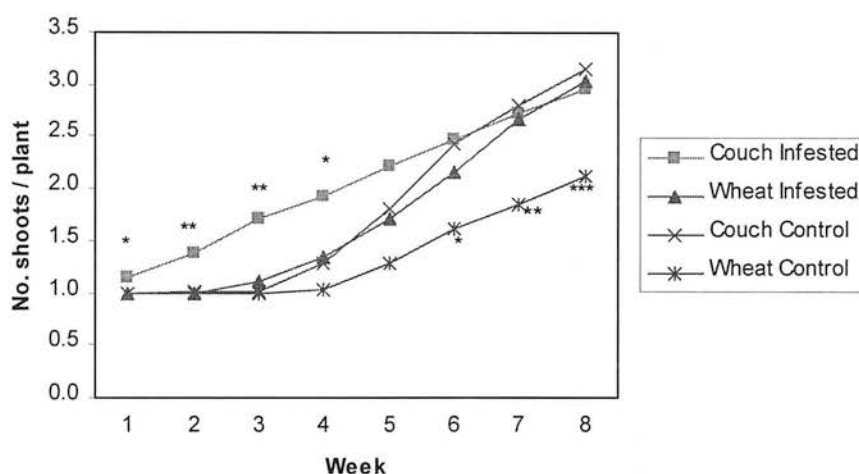


Fig. 4.2b. Effect of WBF larvae on mean number of shoots per plant (No split plots)

Figs. 4.2a and b, and Table 4.1, show that:

- Couch plants had significantly more shoots than wheat plants in weeks 2-4, in week 6, and as a mean over the duration of the experiment ($P < 0.05$).
- Between weeks 2 and 5 and as a mean over the duration of the experiment, plants in infested pots had significantly more shoots than those in control pots. The increase in number of shoots per plant occurred significantly later ($P < 0.05$) in control pots than in infested pots.
- From week 2, plants in the inner ring had significantly more shoots than those in the outer ring. This effect was confirmed by significant differences in all three linear regression parameters. The increase in number of shoots per plant occurred significantly later ($P < 0.05$) in the inner ring than in the outer ring.

- From week 1 to week 4, couch plants in infested pots had significantly more shoots than those in any other treatments. From week 6 to week 8, wheat plants in control pots had significantly fewer shoots than those in any other treatments. These significant interactions between treatment and host plant were, however, due to significant differences between the slopes and shapes of the linear regression curves, rather than differences between their means.
- From week 2 to week 6, couch plants in the inner ring had significantly more shoots than those in any other treatments. This treatment effect was confirmed by significant differences in the overall mean ($P < 0.01$), and in the shape of the linear regression curves ($P < 0.05$).
- From week 2 to week 4, infested plants in the inner ring had significantly more shoots than those in any other treatments.
- In weeks 2 and 3, couch plants in the inner ring of infested pots had significantly more shoots than any other treatments.

Treating clean and infested plants in each pot of the infested treatment as sub-plots produced the following results:

Table 4.2. Effect of WBF larvae on mean number of shoots per plant (Infested or clean plants treated as sub-plots)

Trtmt	Weeks after infestation								Regression parameters		
	1	2	3	4	5	6	7	8	Mean	Lin	Quad
Couch inf	1.29	1.73 *	2.16 *	2.11	2.16	2.31	2.49 c	2.69 c	2.11 b	0.17 c	-0.016 d
Wheat inf	1.00	1.00	1.17	1.27	1.42	1.57	1.76 d	1.85 d	1.38 c	0.13 c	0.0063 c
Couch cln	1.05	1.14	1.23	1.60	2.31	2.92	3.40 b	3.77 b	2.18 b	0.43 b	0.0372 b
Wheat cln	1.00	1.00	1.09	1.46	2.16	3.45	4.71 a	5.96 a	2.6 a	0.73 a	0.1351 a
Sig?	NS	*	*	NS	NS	NS	**	***	**	*	*
Means of host plant effects:											
Couch	1.17	1.44 *	1.70 *	1.86	2.24 *	2.62	2.94	3.23	NSD	0.3	0.011
Wheat	1.00	1.00	1.13	1.37	1.79	2.51	3.24	3.91 *		0.43 **	0.071 *
Means of treatment effects:											
Infested	1.15	1.36 *	1.66 *	1.69	1.79	1.94 **	2.13 ***	2.27 ***	1.75	0.15	-0.0048
Clean	1.03	1.07	1.16	1.53	2.24	3.19	4.05	4.87	2.39 **	0.58 ***	0.0862 ***

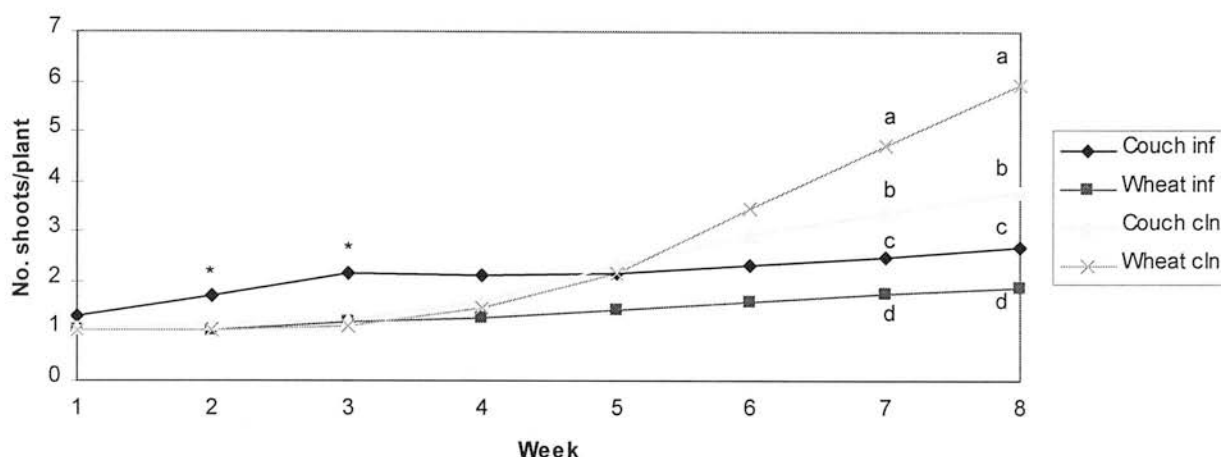


Fig. 4.3. Effect of WBF larvae on mean number of shoots per plant (Infested or clean plants treated as sub-plots)

Fig. 4.3 and Table 4.2 show that:

- Couch plants had significantly more shoots than wheat plants in weeks 2, 3, and 5 ($P < 0.05$). Wheat plants had significantly more shoots than couch plants in week 8 ($P < 0.05$). While there was no significant difference in the overall mean number of shoots on wheat or couch plants, the number of shoots per wheat plant increased more rapidly ($P < 0.01$), and later ($P < 0.05$) than the number of shoots per couch plant.
- Infested plants had significantly more shoots than clean plants in weeks 2 and 3 ($P < 0.05$). Clean plants had significantly more shoots than infested plants from weeks 6 to 8. These treatment effects are strongly supported by significant differences in all three linear regression parameters.
- Infested couch plants had significantly more shoots than any other plants in weeks 2 and 3 ($P < 0.05$). In weeks 7 and 8, there were significant differences between all treatments. Clean wheat plants had the most shoots, followed by clean couch plants, infested couch plants, and infested wheat plants. These interactions between treatment and host plant effects are supported by significant differences in all three linear regression parameters.

4.3.2. Number of shoots killed by WBF larvae

Control pots were disregarded, since none of their shoots or plants were killed by WBF. Considering the inner and outer rings of plants in each pot as sub-plots produced the following results:

Table 4.3. Mean number of shoots per plant killed by WBF larvae

Treatment \ Wk	1	2	3	4	5	6	7	8	Mean
Couch Inner	0.43 a	0.60 *	0.82	1.10	1.42	1.70 *	1.82	1.85	1.22 a
Couch Outer	0.07 b	0.15	0.37	0.60	0.78	1.00	1.12	1.15	0.65 b
Wheat Inner	0.00 c	0.20	0.53	0.60	0.72	0.87	0.88	0.92	0.59 bc
Wheat Outer	0.00 c	0.10	0.30	0.37	0.62	0.82	0.84	0.87	0.49 c
Significance?	***	*	NS	NS	NS	*	NS	NS	*
Means of host plant effects:									
Couch	0.25 *	0.38	0.59 *	0.85 **	1.10 *	1.35 *	1.47	1.50	0.94 *
Wheat	0.00	0.15	0.42	0.48	0.67	0.84	0.86	0.89	0.54
Means of plant ring effects:									
Inner	0.22 ***	0.40 **	0.68 **	0.85 *	1.07 *	1.28 *	1.35 *	1.38 *	0.9 **
Outer	0.03	0.13	0.33	0.48	0.70	0.91	0.98	1.01	0.57

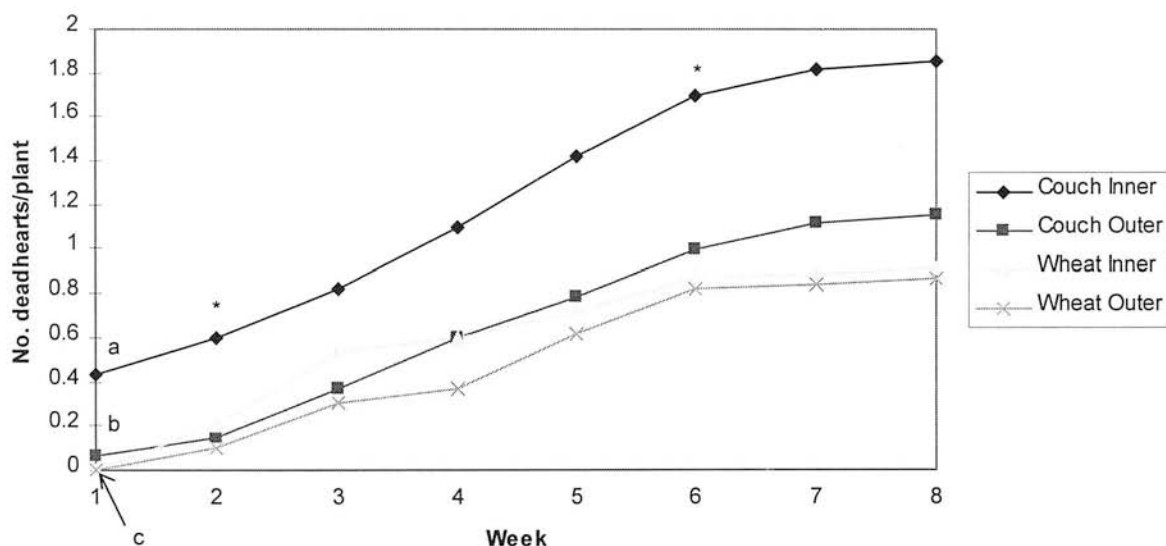


Fig. 4.4. Mean number of shoots per plant killed by WBF larvae

Fig. 4.4 and Table 4.3 show that:

- Couch plants had significantly more deadhearts than wheat plants in weeks 1, 3, 4, 5 and 6.
- The inner ring of plants had significantly more deadhearts than those in the outer ring throughout the experiment.
- Couch plants in the inner ring had significantly more deadhearts than any others in weeks 1, 2 and 6. Furthermore, in week 1, couch plants in the outer ring had significantly more deadhearts than any wheat plants.

- These results are confirmed by significant differences of the overall means between host plants and between treatments, and a significant interaction between these effects. There were, however, no significant differences between other linear regression parameters.

Treating clean and infested plants in each pot of the infested treatment as sub-plots produced the following results:

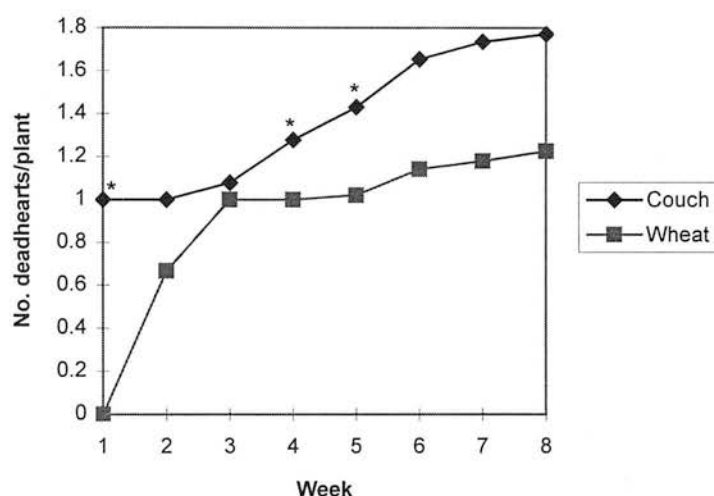


Fig. 4.5. Mean number of shoots per plant killed by WBF larvae (Infested plants only)

Throughout the experiment, WBF larvae killed more couch shoots than wheat shoots, but this effect was only significant ($P < 0.05$) in weeks 1, 4 and 5. This effect was confirmed by a significant difference between the overall means ($P < 0.01$), and between the quadratic parameter of each linear regression curve ($P < 0.01$).

4.3.3. Percentage of shoots killed by WBF larvae

Control pots were disregarded, since none of their shoots or plants were killed by WBF. Considering the inner and outer rings of plants in each pot as sub-plots produced the following results:

Table 4.4. Mean percentage of shoots killed by WBF larvae

Treatment	Weeks after infestation								Regression	
	1	2	3	4	5	6	7	8	Lin	Quad
Couch Inner	35.60***	39.70	42.80	52.20	60.70	67.60	66.70	63.80		-0.0056a
Couch Outer	5.80	12.20	27.20	42.80	54.30	56.90	58.30	58.00	NSD	-0.0031b
Wheat Inner	0.00	20.00	50.60	52.80	58.50	61.90	60.20	61.00		-0.023 c
Wheat Outer	0.00	10.00	27.10	33.80	50.60	61.90	58.50	56.40		-0.0131b
Means of host plant effects:										*
Couch	20.70 **	25.95	35.00	47.50	57.50	62.25	62.50	60.90	No Significant Difference	
Wheat	0.00	15.00	38.85	43.30	54.55	61.90	59.35	58.70		
Means of plant ring effects:										
Inner	17.80***	29.85 **	46.70 *	52.50	59.60	64.75	63.45	62.40	0.065	NSD
Outer	2.90	11.10	27.15	38.30	52.45	59.40	58.40	57.20	0.087 *	

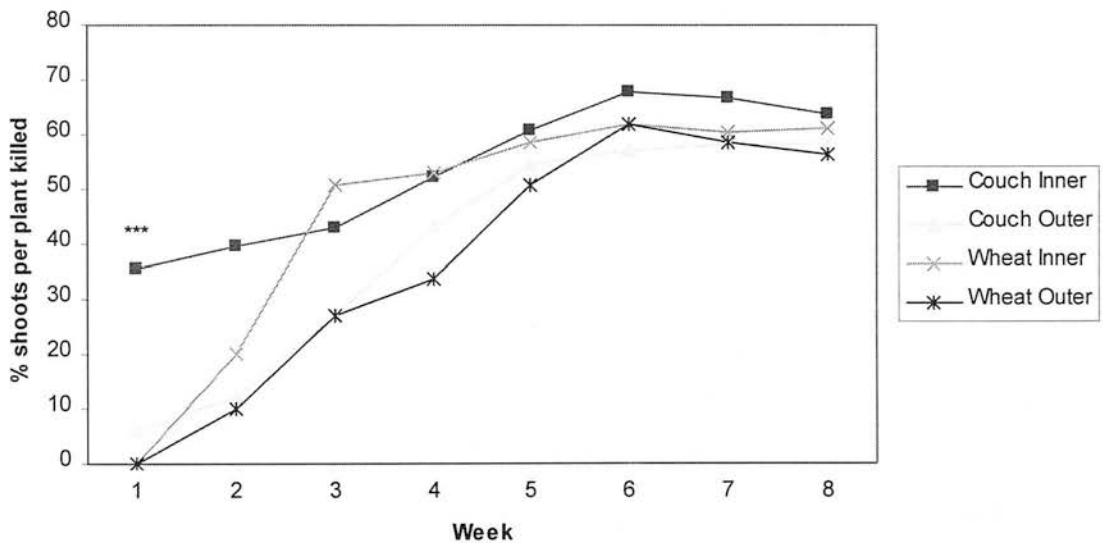


Fig. 4.6. Mean percentage of shoots killed by WBF larvae

Fig. 4.6 and Table 4.4 show that:

- A significantly higher percentage ($P < 0.01$) of couch shoots than of wheat shoots were killed in week 1.
- Until week 3, a significantly greater percentage of shoots were killed in the inner ring than in the outer ring. However, the percentage of shoots killed increased significantly more rapidly in the outer than in the inner ring ($P < 0.05$).

- Couch plants in the inner ring had a significantly higher percentage ($P < 0.001$) of shoots killed than any other plants in week 1. This effect is confirmed by a similar significant difference ($P < 0.05$) in the quadratic parameter of the linear regression curve. The quadratic parameter of the linear regression curve for wheat plants in the inner ring was also significantly different ($P < 0.05$) from all other treatments.

Treating clean and infested plants in each pot of the infested treatment as sub-plots produced the following results:

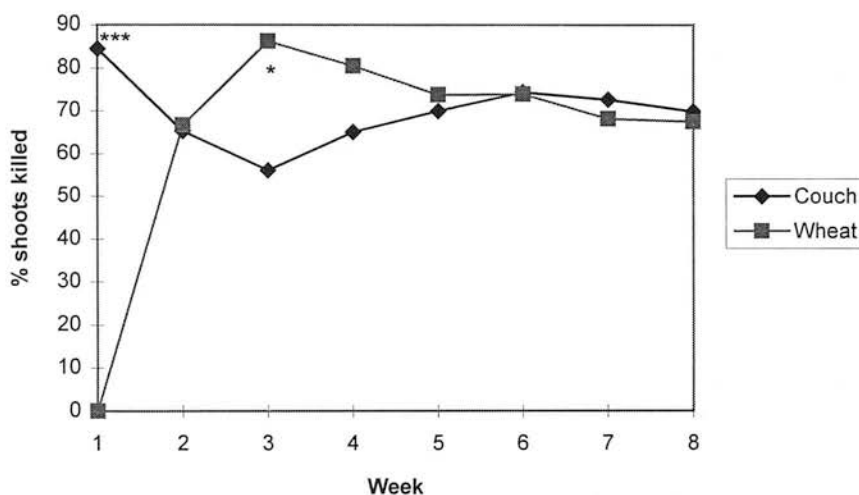


Fig. 4.7. Mean percentage of shoots killed by WBF larvae (Infested plants only)

In week 1, the percentage of couch shoots killed was significantly greater ($P < 0.001$) than the percentage of wheat shoots killed. In week 3, the percentage of wheat shoots killed was significantly greater ($P < 0.05$) than the percentage of couch shoots killed. These effects are confirmed by significant differences in the linear ($P < 0.05$) and quadratic ($P < 0.01$) parameters of the linear regression curves.

4.3.4. Effect of WBF larvae on number of healthy shoots

Considering the inner and outer rings of plants in each pot as sub-plots produced the following results:

Table 4.5. Effect of WBF larvae on mean number of healthy shoots per plant

	Weeks after infestation								Regression parameters		
Treatment	1	2	3	4	5	6	7	8	Mean	Lin	Quad
C If I	0.80	1.03	1.37 a	1.28	1.27	1.18	1.27	1.48			
C If O	0.98	0.98	0.88 b	0.87	0.97	1.05	1.25	1.43			
W If I	1.00	0.80	0.58 c	0.77	1.00	1.33	1.82	2.15			
W If O	1.00	0.90	0.80 b	0.93	1.10	1.28	1.78	2.13		No	
C Ct I	1.00	1.02	1.02 b	1.37	2.02	2.73	3.10	3.42	Significant		
C Ct O	1.00	1.00	1.00 b	1.17	1.58	2.10	2.45	2.88	Differences		
W Ct I	1.00	1.00	1.00 b	1.02	1.37	1.70	2.02	2.35			
W Ct O	1.00	1.00	1.00 b	1.05	1.22	1.53	1.67	1.90			
Significance?	NS	NS	***	NS	NS	NS	NS	NS			
Means of host plant effects:											
Couch	0.95	1.01	1.07	1.17	1.46	1.77	2.02	2.30	No Significant		
Wheat	1.00	0.93	0.85	0.94	1.17	1.46	1.82	2.13	Differences		
Means of treatment effects:											
Infested	0.95	0.93	0.91	0.96	1.08	1.21	1.53	1.80	1.17	0.12	NSD
Control	1.00	1.00	1.00	1.15	1.55 *	2.02 *	2.31	2.64	1.58 **	0.26 **	
Means of plant ring effects:											
Inner	0.95	0.96	0.99	1.11	1.41	1.74	2.05	2.35	No Significant		
Outer	1.00	0.97	0.92	1.00	1.22	1.49	1.79	2.09	Differences		
Host plant / treatment interactions:											
Couch Infested	0.89 *	1.01	1.13 a	1.08	1.12	1.12 c	1.26 c	1.46 c		0.062 c	0.0062 c
Wheat Infested	1.00	0.85 *	0.69 c	0.85	1.05	1.31 c	1.80 b	2.14 b	NSD	0.174 b	0.0542 a
Couch Control	1.00	1.01	1.01 b	1.27	1.80	2.42 a	2.78 a	3.15 a		0.341 a	0.043 ab
Wheat Control	1.00	1.00	1.00 b	1.03	1.29	1.62 b	1.84 b	2.13 b		0.169 b	0.0312 b
Significance?	*	*	**	NS	NS	***	***	***		*	**
Host plant / plant ring interactions:											
Couch Inner	0.90	1.03	1.19 a	1.33 **	1.64	1.96	2.18	2.45			0.014 c
Couch Outer	0.99	0.99	0.94 b	1.02	1.28	1.58	1.85	2.16	No		0.035 b
Wheat Inner	1.00	0.90	0.79 c	0.89	1.18	1.52	1.92	2.25	Significant		0.049 a
Wheat Outer	1.00	0.95	0.90 b	0.99	1.16	1.41	1.72	2.02	Differences		0.036 b
Significance?	NS	NS	**	**	NS	NS	NS	NS			*
Treatment / plant ring interactions:											
Infested Inner	0.90	0.92	0.98	1.03	1.13	1.26	1.54	1.82	No		
Infested Outer	0.99	0.94	0.84	0.90	1.03	1.17	1.51	1.78	Significant		
Control Inner	1.00	1.01	1.01	1.19	1.69	2.22	2.56	2.88	Differences		
Control Outer	1.00	1.00	1.00	1.11	1.40	1.82	2.06	2.39			

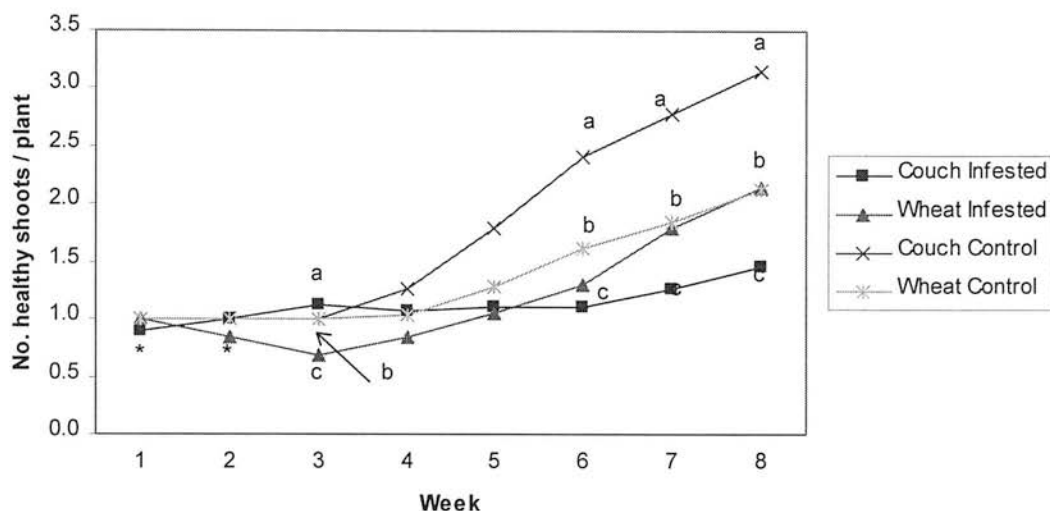


Fig. 4.8. Effect of WBF larvae on mean number of healthy shoots per plant

Fig. 4.8 and Table 4.5 show that:

- Couch plants had no more healthy shoots than wheat plants throughout the experiment.
- Wheat and couch plants in control pots bore significantly more ($P < 0.05$) healthy shoots than those in infested pots in weeks 5 and 6. This effect is confirmed by significant differences between treatments ($P < 0.01$) in the overall means and in the rates of increase of healthy shoot numbers.
- Wheat and couch plants in the inner ring had no more healthy shoots than those in the outer ring throughout the experiment.
- Couch plants in infested pots had significantly fewer healthy shoots than those in any other pots in weeks 1, 7 and 8, and significantly more ($P < 0.01$) than any others in week 3.
- Wheat plants in infested pots had significantly fewer healthy shoots than those in any other treatments in weeks 2 and 3.
- Couch plants in control pots had significantly more healthy shoots than those in any other treatments from week 6 onwards. In addition, in week 6 wheat plants in the control pots had significantly more ($P < 0.001$) healthy shoots than any plants in infested pots.
- Interactions between host plant and treatment effects are confirmed by significant differences in the slopes and shapes of the linear regression curves.

- Couch plants in the inner ring had significantly more ($P<0.01$) healthy shoots than any others in weeks 2 and 3. Wheat plants in the inner ring had significantly fewer ($P<0.01$) healthy shoots than any others in week 3. These interactions are confirmed by similar significant differences in the quadratic parameters of the linear regression curves ($P<0.05$).
- In week 3, infested couch plants in the inner ring had significantly more healthy shoots than any others, while infested wheat plants in the inner ring had significantly fewer healthy shoots than any others ($P<0.001$).

Treating clean and infested plants in each pot of the infested treatment as sub-plots produced the following results:

Table 4.6. Effect of WBF larvae on mean number of healthy shoots per plant (Infested or clean plants treated as sub-plots)

Treatment	Weeks after infestation								Regression parameters		
	1	2	3	4	5	6	7	8	Mean	Lin	Quad
Couch inf	0.29***	0.73	1.08	0.83	0.73	0.66	0.76 c	0.92 c	0.75 c	0.038 c	
Wheat inf	1.00	0.33	0.17 *	0.27	0.40	0.43	0.58 c	0.63 c	0.48 d	-0.005 c	NSD
Couch cln	1.05	1.14	1.23	1.60	2.31	2.92	3.40 b	3.77 b	2.18 b	0.43 b	
Wheat cln	1.00	1.00	1.09	1.46	2.16	3.45	4.71 a	5.96 a	2.6 a	0.73 a	
Sig?	***	NS	*	NS	NS	NS	*	***	*	**	
Means of host plant effects:											
Couch	0.67	0.94	1.16 *	1.22	1.52	1.79	2.08	2.34	NSD	0.23	0.0094
Wheat	1.00 *	0.67	0.63	0.87	1.28	1.94	2.65 *	3.29**		0.36 *	0.0889**
Means of treatment effects:											
Infested	0.65	0.53	0.62	0.55	0.57	0.54	0.67	0.77	0.61	0.016	0.012
Clean	1.03***	1.07**	1.16**	1.53***	2.24***	3.19***	4.05***	4.87***	2.39***	0.578***	0.086***

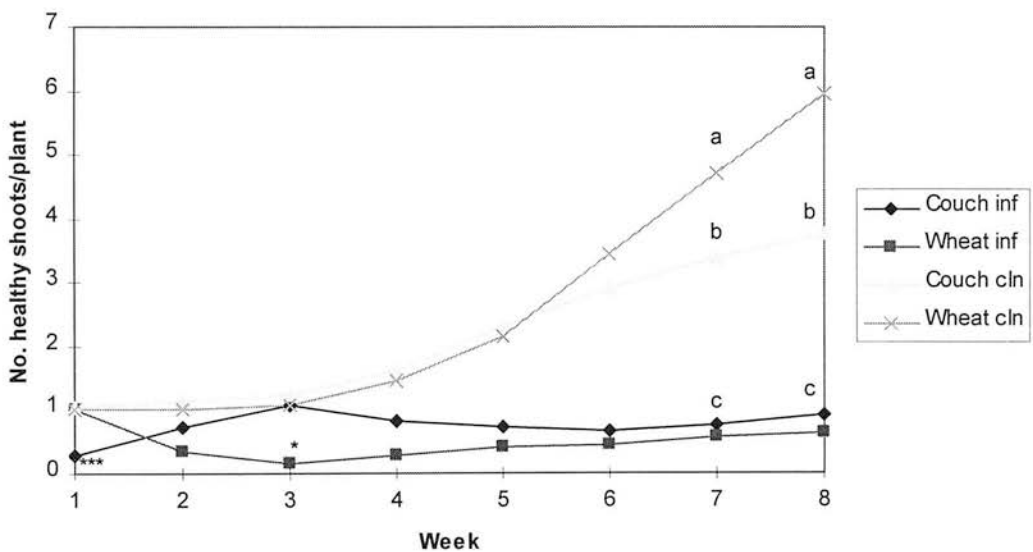


Fig. 4.9. Effect of WBF larvae on mean number of healthy shoots per plant (Infested or clean plants treated as sub-plots)

Fig. 4.9 and Table 4.6 show that:

- Wheat plants had significantly more healthy shoots than couch plants in weeks 1, 7 and 8. Couch plants had significantly more ($P<0.05$) healthy shoots than wheat plants in week 3. The number of healthy shoots increased significantly more rapidly ($P<0.05$) in wheat than in couch, but this effect was significantly delayed ($P<0.01$).
- Clean plants of both species had significantly more healthy shoots than infested plants throughout the experiment. This effect is supported by significant differences in all three parameters of the linear regression curves of both treatments ($P<0.001$).
- Infested couch plants had significantly fewer ($P<0.001$) healthy shoots than any others in week 1.
- Infested wheat plants had significantly fewer ($P<0.05$) healthy shoots than any others in week 3.
- In weeks 7 and 8, clean wheat plants had significantly more healthy shoots than any others, and clean couch plants had significantly more healthy shoots than any infested plants. This effect is supported by significant differences in the overall means and linear parameters of the linear regression curves.

4.3.5. Effect of WBF larvae on number of leaves per plant

Throughout the experiment, there was no significant difference between the mean number of leaves on plants in the inner and outer rings. There was, however, a significant difference ($P < 0.05$) between the quadratic parameters of the linear regression curves for the inner and outer rings. This indicates that the number of leaves on plants in the inner rings increased later than on plants in the outer rings.

Table 4.7. Effect of WBF larvae on mean number of leaves per plant

		Weeks after infestation								Regression parameters		
Treatment	1	2	3	4	5	6	7	8	Mean	Lin	Quad	
Couch inf	1.23	1.52 a	1.63	1.79	1.98	2.01	2.34 d	2.83 d		0.2 c	0.015 c	
Couch cntrl	0.78	1.44 a	1.87	2.41	3.03	3.93	5.55 a	6.76 a	NSD	0.82 a	0.09 a	
Wheat inf	0.35	0.70 c	1.08 *	1.47	1.78	2.28	2.81 c	3.88 c		0.46 b	0.04 b	
Wheat cntrl	0.03	0.98 b	1.77	2.16	2.98	3.58	4.51 b	6.06 b		0.79 a	0.038 b	
Significance?	NS	*	*	NS	NS	NS	**	**		*	*	
Means of host plant effects:												
Couch	1.00 **	1.48 *	1.75	2.10	2.50	2.97	3.95	4.80	No significant differences			
Wheat	0.19	0.84	1.42	1.81	2.38	2.93	3.66	4.97				
Means of treatment effects:												
Infested	0.79	1.11	1.35	1.63	1.88	2.15	2.58	3.36	1.85	0.33	0.028	
Control	0.40	1.21	1.82 *	2.28 ***	3.00 ***	3.75 **	5.03 **	6.41 **	2.99 ***	0.81 ***	0.064 *	

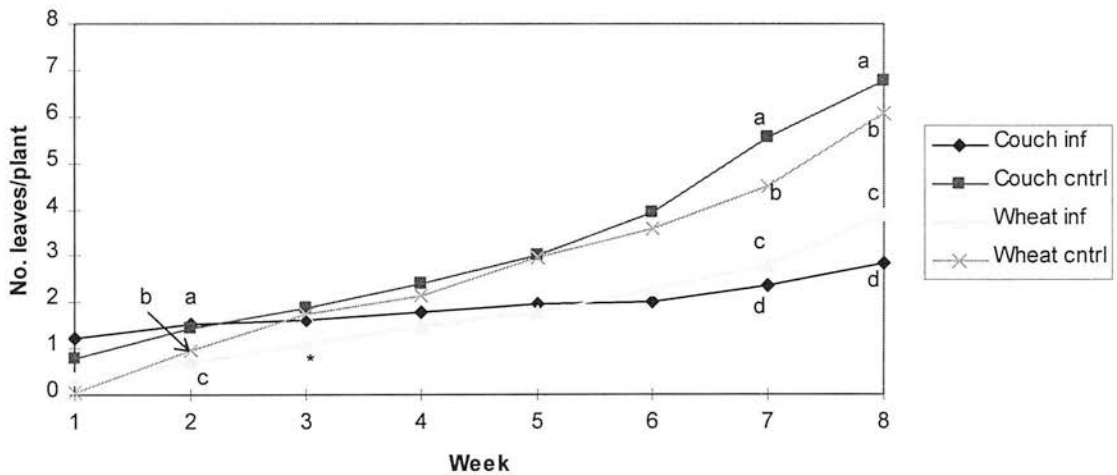


Fig. 4.10. Effect of WBF larvae on mean number of leaves per plant

Fig. 4.10 and Table 4.7 show that:

- Couch plants had significantly more leaves than wheat plants in weeks 1 and 2

- Plants of both species in control pots had significantly more leaves than those in infested pots from week 3 onwards. This is borne out by significant differences in all 3 linear regression parameters.
- Wheat plants in infested pots had significantly fewer leaves than those in any other treatments in weeks 2 and 3. Furthermore, wheat plants in control pots had significantly fewer leaves than any couch plants in week 2. ($P < 0.05$)
- In weeks 7 and 8, there were significant differences between all treatments ($P < 0.01$). Couch plants in control plots had the most leaves, followed by wheat plants in control pots, wheat plants in infested pots, and couch plants in infested pots. These effects are borne out by significant differences ($P < 0.05$) in the linear and quadratic parameters of the linear regression curves.

Treating clean and infested plants in each pot of the infested treatment as sub-plots produced the following results:

Table 4.8. Effect of WBF larvae on mean number of leaves per plant (Infested or clean plants treated as sub-plots)

Treatment	Weeks after infestation								Regression parameters		
	1	2	3	4	5	6	7	8	Mean	Lin	Quad
Couch inf	1.10	1.13 a	1.10	1.32	1.39	1.28	1.49	1.72 c		0.081c	0.01 c
Wheat inf	0.37	0.40	0.39	0.51	0.90	1.07	1.18	1.42 c	NSD	0.163c	0.016 c
Couch cln	1.28	1.78	2.32	2.64	3.58	4.72	5.55	7.11 b		0.807b	0.082 b
Wheat cln	0.35	0.81	1.56	2.36	3.47	5.65	7.03	10.02a		1.336a	0.177 a
Sig?	NS	NS	NS	NS	NS	NS	NS	*		*	*
Means of host plant effects:											
Couch	1.19 *	1.45 *	1.71 *	1.98	2.48	3.00	3.52	4.42	NSD	0.44	0.046
Wheat	0.36	0.60	0.97	1.44	2.19	3.36	4.10	5.72 *		0.75 **	0.096 *
Means of treatment effects:											
Infested	0.73	0.76	0.74	0.92	1.15	1.17	1.33	1.57	1.05	0.12	0.013
Clean	0.81	1.29 **	1.94***	2.50 ***	3.52***	5.19***	6.29***	8.56***	3.76**	1.07 **	0.13 *

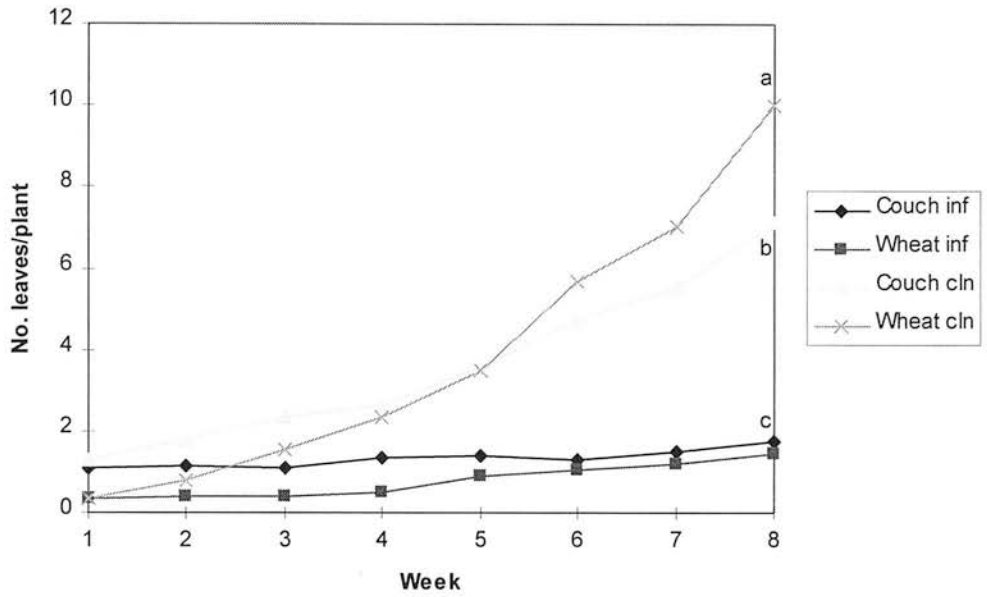


Fig. 4.11. Effect of WBF larvae on mean number of leaves per plant (Infested or clean plants treated as sub-plots)

Fig. 4.11 and Table 4.8 show that:

- Couch plants had significantly more ($P < 0.05$) leaves than wheat plants until week 3. Wheat plants had significantly more leaves ($P < 0.05$) than couch plants in week 8. This effect is borne out by significant differences in the linear and quadratic parameters of the linear regression curves.
- Clean plants of both species had significantly more leaves than infested plants from week 2 ($P < 0.01$) onwards ($P < 0.001$). This effect is borne out by significant differences in all 3 parameters of the linear regression curves.
- In week 8, clean wheat plants had significantly more leaves than any others, and clean couch plants had significantly more leaves than any infested plants ($P < 0.05$). Similar significant differences ($P < 0.05$) are found in the linear and quadratic parameters of the linear regression curves.

4.3.6. Number of plants killed by WBF larvae

The number of plants in infested pots killed by WBF, considering the inner and outer rings of plants as split plots, is presented in Fig. 4.12 below. The data were highly variable, so despite appearances, the only significant effect is that more wheat plants than couch plants were killed in week 5 ($P < 0.05$).

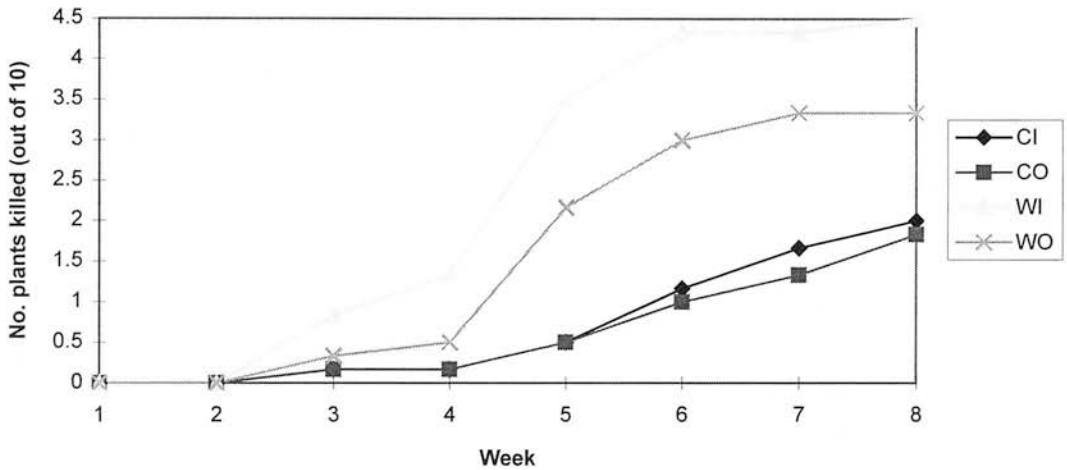


Fig. 4.12. Mean number of plants killed by WBF larvae

4.3.7. Summary of effects of WBF larvae on host plants

Host plants respond to the early stages of wheat bulb fly infestation by producing extra shoots, especially in weeks 2-5 (Fig. 4.2 and Table 4.1), but these extra shoots are killed by larvae, leaving no more healthy shoots in infested pots than in control pots (Fig. 4.8, Table 4.5).

From week 5, infested plants suffer a relative reduction in number of shoots. This reduction, when all the plants in a pot are considered, is only significant in weeks 5 and 6 (Table 4.5). However, if clean and infested plants within infested pots are considered separately, clean plants compensate for shoot death in infested neighbours by producing more shoots themselves. In weeks 7 and 8, this effect is especially marked in wheat (Table 4.2, Fig. 4.9, Table 4.6).

Plants in the inner ring produce more shoots (Table 4.1), but once again, extra shoots are killed by WBF larvae (Tables 4.3, 4.5). Plants in the inner ring are the first to be attacked, and there is no significant difference in the number of healthy shoots in the inner and outer rings, so this would not appear to be related to any other differences in growth conditions.

Between weeks 2 and 6, couch plants produced more shoots than wheat plants (Fig. 4.2 and Table 4.1). However, these extra shoots are mostly produced by infested plants (Table 4.1), and are killed by WBF larvae (Table 4.3), so that there is no significant difference between the number of healthy shoots on couch and wheat plants (Table 4.5).

In week 1, no wheat shoots were killed, but by week 3, almost as many wheat shoots as couch shoots were dead (Table 4.3, Fig. 4.4). In week 3, a higher percentage of shoots were killed on infested wheat plants than on infested couch plants (Table 4.6). The number of shoots killed in the inner ring of couch plants is consistently highest throughout the experiment, and significantly highest in weeks 1, 2 and 6; this would suggest that larval food demand is highest in the early stages of infestation, and is more satisfied in couch than in wheat.

It is apparent from Figs. 4.10 and 4.11, and from Tables 4.7 and 4.8 that WBF infestation limits the number of leaves that couch or wheat plants can produce, and that infested plants do not recover for at least 8 weeks. This would limit their photosynthetic potential; thus we can conclude that, in these experimental conditions, neither plant is particularly well-adapted to WBF infestation.

4.3.8. Mortality and development rate of wheat bulb fly larvae and pupae

The numbers of adult flies per infested pot, and the mean number of days to their eclosion are presented in Table 4.9, and graphically represented in Fig. 4.13. Due to the high mortality rates of all flies, Kaplan-Meier survival analysis in SPSS showed no significant differences in survival, but the date of 75% eclosion was significantly earlier in couch-raised males than in wheat-raised females (Fig. 4.14). The trends in both interpretations agreed, so this interesting phenomenon was further examined while rearing WBF for oviposition bioassays in April and May 2000 (See Chapter 5).

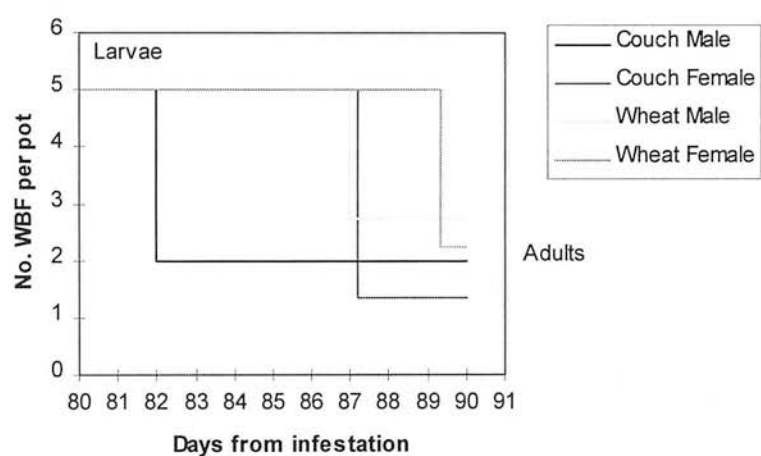


Fig. 4.13. Survival of WBF larvae and pupae

Table 4.9. Survival of WBF larvae and pupae

Reared on:	Sex	Adults /pot	Days to eclosion
Couch	Male	2	82
	Female	1.33	87.2
Wheat	Male	2.75	87
	Female	2.25	89.3

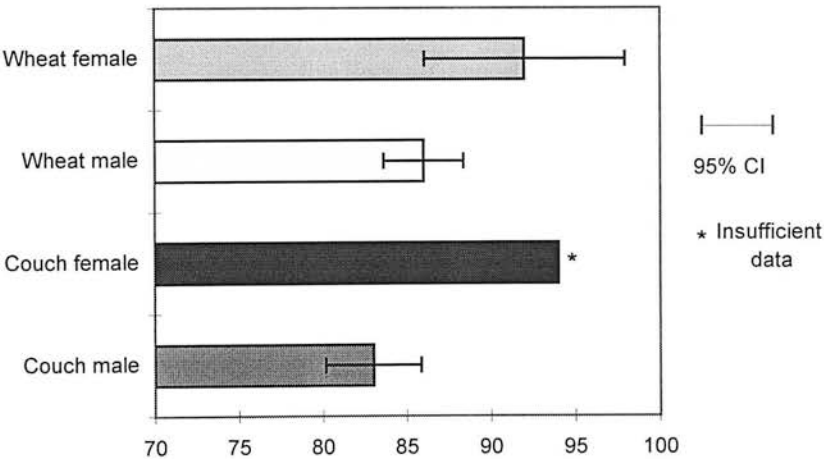


Fig. 4.14. Kaplan-Meier survival analysis
Days to 75% eclosion of adult WBF

4.3.9. Fecundity of adult wheat bulb flies

There were not enough eggs laid by gravid female flies to provide a meaningful comparison of the fecundity of couch-reared and wheat-reared flies.

4.4. Conclusions and discussion

Couch and wheat respond differently to attack by WBF larvae. Both produce extra shoots in the early infestation, although this response is more marked and more rapid in couch than in wheat. However, these extra shoots are soon consumed by the developing larvae, and after 5 weeks, infested plants of both species have fewer shoots than clean plants. WBF larvae are more likely to kill wheat plants than couch plants, but clean wheat plants are better able than clean couch plants to compensate for the death of neighbouring infested plants, presumably through reduced competition for light, water and nutrients. Thus compensation for WBF attack occurs both within plants, as reported by Long and Morris (1961), and between plants, as reported by Bardner *et al.* (1969). Within-plant compensation is more marked in couch than in wheat, and inter-plant compensation is more marked in wheat than in couch. However, within-plant compensation for WBF infestation is not complete in either species, as shown by the reduced number of leaves per infested plant, and hence, reduced photosynthetic potential.

However, the couch plants in this experiment were grown from the shortest possible length of rhizome, with only one node. Couch plants in the field would, on average, grow from longer sections of rhizome with several nodes, and might thus recover better from wheat bulb fly attack at one node, by compensatory growth at other nodes. Gemmill (1927) found WBF larvae feeding on the rhizomes as well as the shoots of couch grass. One could speculate that a larva which kills all the shoots on the apical node of a couch rhizome would simply eat along the rhizome to the next node, which would already be producing more shoots due to the removal of apical dominance.

Jones (1978) found the weight and development rate of WBF larvae and pupae varied greatly with temperature. Long (1960c) reported that the size of mature WBF larvae

varied considerably, and that smaller adults produced fewer eggs. It would thus be very interesting to measure the effect of host plant on larval weight, and hence, perhaps, adult fecundity. However, this would have damaged both larva and host plant, and in the present study both were needed for further rearing and experiments. Although the present study found no difference in WBF survival rates when raised on wheat or couch, survival is not the only measure of insect fitness.

Similarly, Griffiths and Scott (1969) found that three-leaf wheat plants on which live WBF larvae had fed weighed less after 48 days than undamaged plants. Numbers of shoots and leaves are not the only measures of host plant fitness, especially in perennial grasses such as couch. Further studies should investigate the effect of WBF infestation on biomass and resource partitioning of host plants.

If the apparent earlier eclosion of WBF adults on couch than on wheat proves statistically significant (Fig. 4.14), one could conclude that WBF larvae develop more rapidly (as reported by Gemmill, 1927) and use more resources, on couch than on wheat, i.e. that they are better adapted to couch as a food source. Earlier eclosion in the field would allow adults to make better use of favourable weather conditions, and to live longer, mate more often, and produce more eggs. Cooper (1978) found that, in 1975, when emergence of adult WBF started 8 days later than in 1976 and 1977, fewer flies produced a second batch of eggs.

Chapter 5

Survival and development rate of wheat bulb fly larvae and pupae

Chapter 5. Survival and development rate of wheat bulb fly larvae and pupae

5.1. Introduction

Jones and Moore (1978) achieved their highest survival rates (55-70%) of wheat bulb fly (WBF) larvae and pupae when neonate larvae were placed on 3- or 4-leaved wheat shoots, then kept for three weeks at 10°C/12hr nights, followed by four or more weeks at a constant 20°C for a 12 hour day. Pot trials in 1999 under similar conditions indicated that WBF adults may emerge earlier when raised on couch than when raised on wheat, although these results were only significant when comparing 75% eclosion of couch-raised males and wheat-raised females (Chapter 4). Between January and May 2000 this experiment was modified and repeated on a larger scale. The objective was to determine whether WBF larvae and pupae had higher survival rates and developed more rapidly when raised on couch instead of wheat.

5.2. Materials and methods

Two hundred untreated wheat seeds, cv. Mercia, were sown 2cm deep in John Innes No. 3 compost over gravel in a plastic crate 30 x 55 x 38cm deep, with drainage holes drilled in the bottom, and placed in a growth room at a constant 15°C, with a 12hr day. After 2 weeks, by which time most of the seedlings had emerged and were about to produce their first leaves, active neonate WBF larvae from the bioassays described in Chapter 3 were added daily to the crate over the next week. Two more replicates were treated in the same way, starting at weekly intervals.

Four hundred untreated couch seeds (Herbiseed) were sown just below the surface of compost in identical crates. They were left in a Fison's growth cabinet at 10°C/12hr nights, 25°C/12hr days for 3 weeks, as recommended by the suppliers (Herbiseed, pers. comm.), by which time most of the seedlings emerged and were about to produce their first leaves. Active neonate WBF larvae from the bioassays described

in Chapter 3 were added to the crate, which was transferred to the growth room (constant 15°C, with a 12hr day) where the wheat seeds had germinated. Three more replicates were treated in the same way, starting at weekly intervals.

Thereafter, crates of couch and wheat plants were treated identically. After inoculation with WBF larvae, they were kept in the 15°C growth room for 3 weeks, then transferred to cages in a growth room kept at a constant 20°C for a 12 hour day. Each crate was kept in a nylon mesh cage 45cm wide x 70cm x 1m high. The front, narrow side was fastened with Velcro™, and included a 12cm diameter hole closed with a stocking, allowing easy access to remove adult flies with an aspirator. After 8 weeks, adult flies started to emerge and the cages were checked daily; their sex and numbers, and the date were recorded, and they were transferred to a rearing cage for use in oviposition experiments.

The crates of compost were soaked with water at the start of the experiment, and thereafter watered with an overhead spray twice weekly.

5.3. Results

Data were collated and analysed in Excel 5.0.

Mortality rates were highly variable, ranging from 26.8% to 74.5%, and it was immediately apparent that these differences did not depend on sex of the adult fly or host plant of the larva.

However, it was also apparent that larvae added to host plants at a later date developed more rapidly. Since, for logistical reasons, the four couch replicates had been started before the three wheat replicates, this effect masked any difference in eclosion times according to host plant. Accordingly, the results were analysed using a multiple regression of days to eclosion vs. host plant species and estimated age of eggs at hatching. WBF oviposition usually peaks in August (Oakley & Uncles, 1977;

Cooper, 1978), so 15th August was taken as the nominal date of egg laying. These results are shown, together with regression lines (predicted days to eclosion) in Figs. 5.1. and 5.2., below. Male and female adults were considered separately, since males emerge about a week earlier than females (Gemmill, 1927, and many subsequent authors).

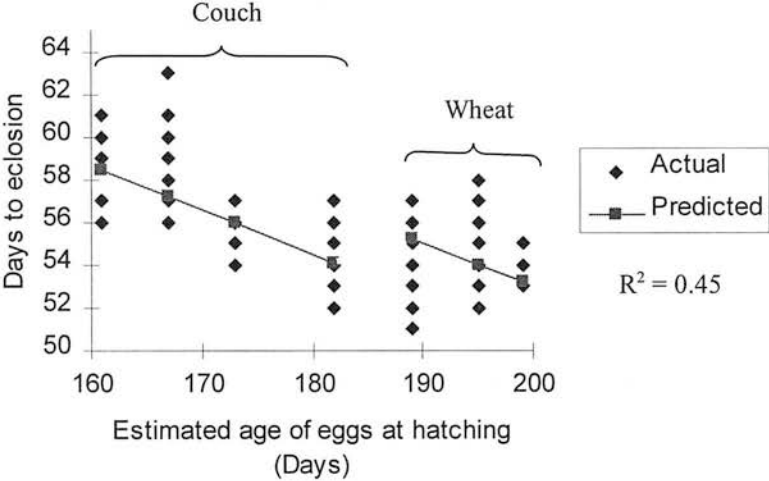


Fig. 5.1. Effect of host plant and age of eggs on male WBF development

The regression equation for days to male WBF eclosion is:

$$\begin{array}{rcl} \text{Days to eclosion} & = & 88.79 - 0.20(\text{Age of eggs}) + 2.55(\text{Host plant}) \\ \text{Standard Error} & & 3.20 \quad 0.02 \quad 0.46 \end{array}$$

where Couch = 1, Wheat = 2.

Thus, male WBF raised on couch emerge 2.55 days earlier than those raised on wheat ($P < 0.001$), and for every extra day since oviposition, larval and pupal development time is reduced by 0.2 days ($P < 0.001$).

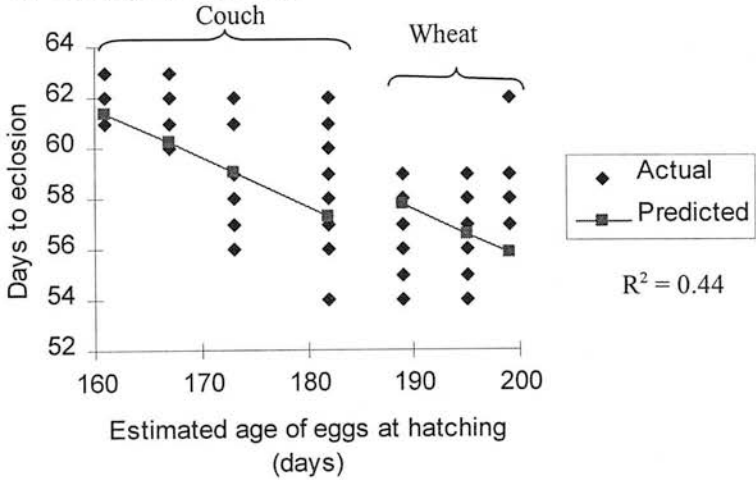


Fig. 5.2. Effect of host plant and age of eggs on female WBF development

The regression equation for female WBF eclosion time is:

$$\begin{array}{lcl} \text{Days to eclosion} = & 90.83 - 0.19(\text{Age of eggs}) + 1.81(\text{Host plant}), \\ \text{Standard Error} & 3.60 \quad 0.02 \quad 0.51 \end{array}$$

where Couch = 1, Wheat = 2.

Thus, female WBF raised on couch emerge 1.81 days earlier than those raised on wheat ($P < 0.001$), and for every extra day since oviposition, larval and pupal development time is reduced by 0.19 days ($P < 0.001$).

5.4. Conclusions and discussion

These results show that WBF adults emerge earlier when reared on couch than when reared on wheat. This accords with Gemmill's statement (1927) that "a newly-hatched larva can complete its life-history, up to the emergence of the fly from the pupa, rather more quickly (8 weeks) even than in wheat (8½ to 9 weeks)". This would account for their choice of couch over wheat as larvae (See Chapter 3), and may be due to their higher consumption of couch shoots than wheat shoots in the early stages of infestation (See Chapter 4). Earlier eclosion, even by a few days, would allow more time for mating and egg-laying, and thereby increase fecundity. Long (1958c) found that, in the laboratory, the rate of egg-laying increased with the age of gravid female WBF; if this effect were repeated in the field, it would provide another advantage to earlier eclosion.

These findings provide further evidence to support the central hypothesis that couch is the primary host of wheat bulb fly.

It was intended to compare the fecundity of couch-reared and wheat-reared WBF, but nearly all the couch-reared WBF adults died due to over-heating of the growth room in which they were reared. Consequently, this part of the study was abandoned. As mentioned in Chapter 4, Long (1960c) found that the size of mature WBF larvae varied considerably, and that smaller adults produced fewer eggs. In the present study, WBF larvae and pupae were not weighed, since the insects and their hosts

were needed for further rearing and experiments. Ideally, the number of eggs laid by couch-reared and wheat-reared WBF females would be compared, and related to larval and pupal weights.

The more rapid development of larvae and pupae from older eggs has not been reported before, and was entirely unexpected. Way (1959) suggested that embryos in WBF eggs normally become fully-developed between egg-laying and the onset of diapause, although diapause can begin before morphological development is complete and gradually slow it down, finally stopping it. Jones and Moore (1978) found that highest rates of egg hatch occurred when eggs were stored for two months at 15°C, followed by two months at 0°C. Both papers (Way, 1959; Jones & Moore, 1978) evaluated optimal egg storage conditions in terms of percentage egg hatch, but not in terms of subsequent larval and pupal development rates.

In the present study, the age of eggs at hatching had five components: time from laying until sampling; time from sampling until extraction from soil, when soil samples were kept in sealed plastic bags in the laboratory; time stored at 15°C, approximately two months; time stored at 5°C, approximately two months; post-diapause time between removal from storage and hatching. The first two components were highly variable, and the first can only be estimated. The other components were much less variable, but it should be stressed that 5°C is higher than the optimal temperature of 0°C for completion of diapause, as determined by Way (1959), and Jones and Moore (1978). Furthermore, Way (1959) found that, at temperatures above the optimum, more time was needed for eggs to complete diapause. More detailed analysis of which of these five components were responsible for the observed effect of accelerated larval and pupal development would be over-interpreting the available data. Such analysis should await further experiments properly designed to study this effect. A further complicating factor is Long's (1960c) observation that adult flies emerge earlier from smaller pupae; as discussed in Chapter 4, the effect of host plant on larval and pupal weight is not known. Nevertheless, one can hypothesise that earlier-laid eggs would develop more fully

before diapause, and would need less time to develop as larvae. If this hypothesis could be proven in further research, it would add further weight to the importance of early eclosion and hence earlier egg-laying. In order to be certain about the date of egg-laying, such research would have to rely on laboratory-reared eggs, rather than eggs collected from the field.

Embryonic diapause is comparatively rare in the Diptera; exceptions include several Culicidae (Saunders, 1982) and *Delia fabricii* Holm. (Johansen, 1990), a close relative of WBF found in northern Norway. The phenomenon of earlier-laid eggs completing larval and pupal development more rapidly has not been reported in any close relation of WBF, although Carriere, Simons and Roff (1996) found the opposite effect in the univoltine cricket *Gryllus pennsylvanicus* Burmeister.

Chapter 6

Plant choice in adult wheat bulb flies

Chapter 6. Plant choice in adult wheat bulb flies

6.1. Introduction

A comparison of the numbers of wheat bulb fly (WBF) adults resting on couch plants and wheat plants might provide interesting supporting evidence as to which plant is the insect's natural host. Adult WBF have frequently been found resting on couch plants in the field (e.g. Petherbridge, 1921). In a survey in eastern Canada and the north-eastern United States, McAlpine and Slight (1981) "found that the adults of *D. coarctata* are strongly associated with the heads and stems of couch grass" and stated that "the primary host [of WBF] is couch grass". It is interesting to note that the first documented occurrence of WBF in North America was in 1954, but it has yet to be reported there as a serious pest of winter wheat.

Jones (1970b) established that adult WBF feed on the saprophytic organisms, especially *Septomyxa affinis* Sherb., found on the dead leaves and ears of grasses, and on the honeydew produced by aphids feeding on grasses. They sheltered within wheat crops during the day, but moved to the ears in the late afternoon. Long (1958d) concluded that adult WBF, especially females, frequently foraged for food during the day but returned to the wheat crop in the evening. Gough (1946) found large field aggregations of mostly male flies at about the time of mating.

6.2. Adult wheat bulb fly plant choice in the laboratory

6.2.1. Materials and methods

Hopkins (1994) used a turntable apparatus developed by Ellis and Hardman (1975) for testing the host plant preferences of cabbage root fly (*Delia radicum* L.). While this method gives good control for factors such as plant position and lighting, it requires at least 50 adult flies per replicate. The numbers of flies raised for the present study would not permit this, so the method adopted here follows that used by Degen, Städler and Ellis (1999) for carrot flies (*Psila rosae* F.).

Adult flies emerging from pots used in the trial described in Chapter 4 were transferred to one of two 60 x 60 x 120cm high cages in the glasshouse, according to whether they had been raised on couch or on wheat plants. Eight 12cm plant pots filled with compost were arranged around the internal sides of the cage floor, four containing a single wheat plant, and four containing a single couch plant, arranged alternately. In the centre of each cage a perspex shelf was balanced on the pots, and on this were four 9cm diameter Petri dishes. These contained, respectively, sterile distilled water, 1g milk powder and 1g yeast powder in 10ml water, citrated sheep's blood (donated by Diagnostics Scotland) diluted to 50% with water, and a 10% solution of honey in water, as recommended by Jones and Moore (1978). In each Petri dish the liquid was absorbed by a dental wick (Kent Dental Supplies) or, in the case of the water, cotton wool. These dishes were changed twice a week, and 5ml sterile distilled water was added to each on intervening days.

Every afternoon for the following 7 weeks, the number of flies was recorded on each plant, on each wall of the cage, on the roof of the cage, and on the food shelf. Flies generally remained static unless disturbed by the observer moving suddenly or casting a shadow on the cage. Counting continued until all visible flies had been accounted for, but some remained hidden during observation. Initial attempts to account for all the flies in each cage were not successful, and only resulted in disturbance of the observed flies. A total of 45 couch-reared flies, and 49 wheat-reared flies were observed. After counting, each pot and each food dish was moved clockwise one place, and the sides and roofs of the cage were sprayed with water.

6.2.2. Results

In both cages, flies appeared to prefer resting on the roofs and the upper parts of the walls. Even on days when flies were recorded resting on plants (32 days for couch-reared flies, and 33 days for wheat-reared flies), only 23% of the couch-reared and 30% of the wheat-reared flies did so.

Analysis of variance treating wheat plants and couch plants as separate treatments, and each day when flies were observed on plants as a separate replicate, showed that, in both cages, adult WBF significantly preferred to rest on couch plants than on wheat plants (Table 6.1, overleaf).

Table 6.1. Plant choice of adult wheat bulb flies

Flies raised on:	Mean daily no. flies found on:		SED	P
	Couch	Wheat		
Couch	2.09	0.34	0.10	<0.001
Wheat	3.55	0.79	0.16	<0.001

However, if each plant is considered as a separate treatment in the analysis of variance, a more complex picture emerges (Table 6.2 and 6.3, Figs. 6.1 and 6.2). In both tables and both figures, different letters denote a significant difference between means ($P < 0.05$).

Table 6.2. Plant choice of couch-reared adult WBF

	Mean no. flies found on plant			
	1	2	3	4
Couch	0.38 ^b	0.47 ^b	0.66 ^a	0.59 ^a
Wheat	0.03 ^c	0.09 ^c	0.13 ^c	0.09 ^c

SED = 0.10, $P < 0.05$

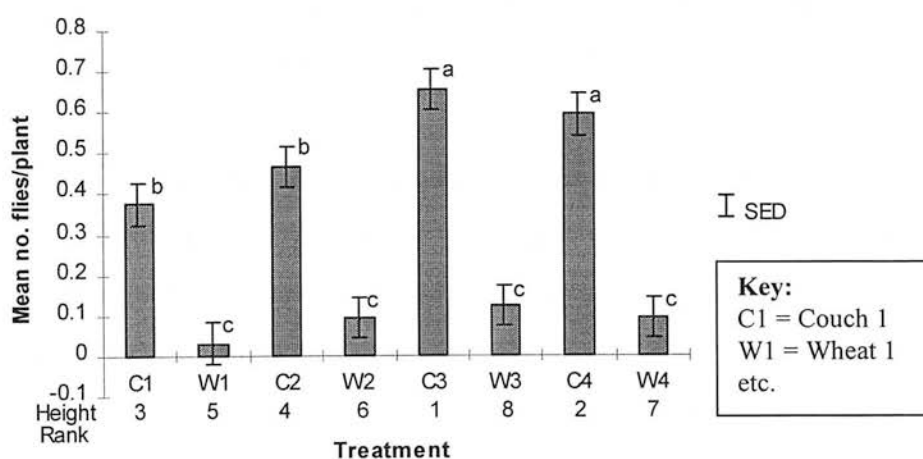


Fig. 6.1. Plant choice of couch-reared adult WBF

Couch-reared WBF significantly chose plants Couch 3 and Couch 4 above all others, and plants Couch 1 and Couch 2 above all wheat plants ($P < 0.05$). There was no significant choice between wheat plants.

Table 6.3. Plant choice of wheat-reared adult WBF

	Mean no. flies found on plant			
	1	2	3	4
Couch	0.64 ^b	0.39 ^c	1.21 ^a	1.30 ^a
Wheat	0.24 ^{cde}	0.09 ^e	0.30 ^{cd}	0.15 ^{de}

SED = 0.16, $P < 0.05$

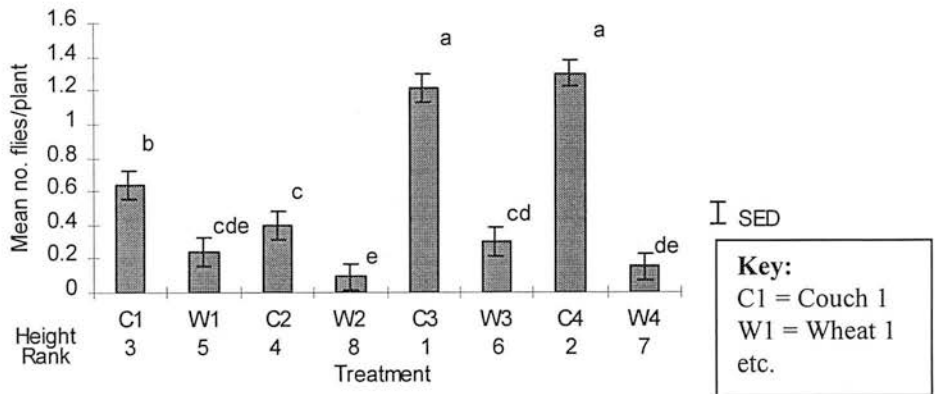


Fig. 6.2. Plant choice of wheat-reared adult WBF

Wheat-reared WBF significantly chose plants Couch 3 and Couch 4 above all others ($P < 0.05$). There was no significant choice of plant Couch 2 over plants Wheat 1 or 2. Plant Wheat 3 was significantly chosen above Wheat 2 ($P < 0.05$).

In both cages, plants differed markedly in height. Height ranks of plants are shown in Figs. 6.1 and 6.2. From these, it appears that there may be some association between plant choice and plant height, so for both groups of insects a multiple regression was performed of mean no. flies/plant against plant height rank and plant species, where Couch = 1 and Wheat = 2. The results are shown in Figs 6.3 and 6.4, overleaf.

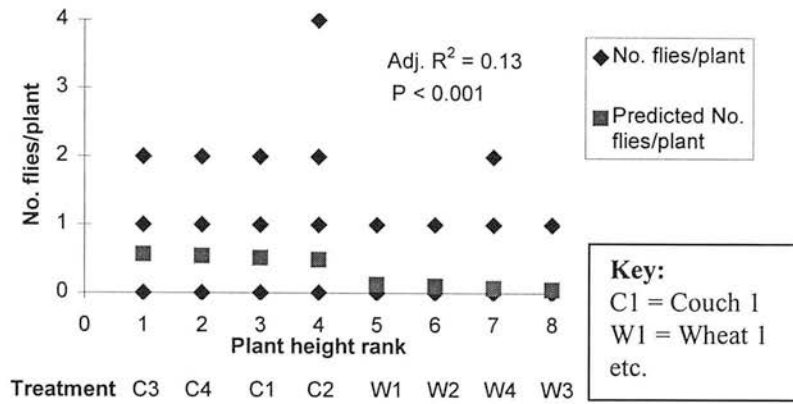


Fig. 6.3. Effect of plant height on plant choice of couch-reared adult WBF

For couch-reared WBF,

$$\begin{aligned} \text{Mean no. flies/plant} &= 0.92 - 0.34(\text{Plant species}) - 0.03(\text{Plant height rank}) \\ \text{Standard Error} &\quad 0.12 \quad 0.15 \quad 0.03 \end{aligned}$$

The relationship with plant species is significant ($P < 0.05$), but the relationship with plant height rank is not significant at all ($P = 0.43$). This implies that couch-reared WBF chose couch plants to rest upon, on the basis of plant species rather than plant height.

However, for wheat-reared flies, a different picture emerges:

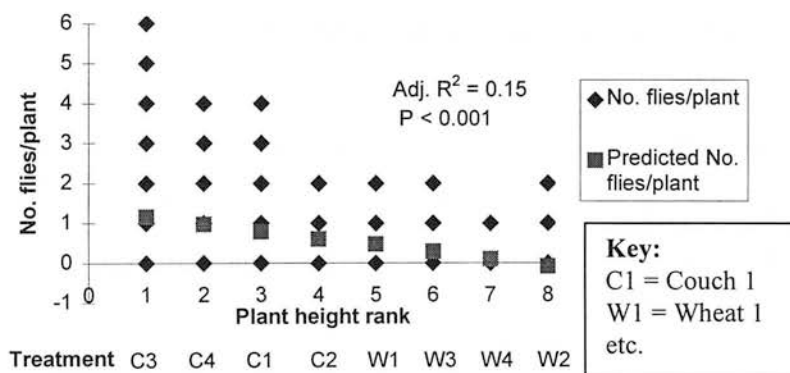


Fig. 6.4. Effect of plant height on plant choice of wheat-reared adult WBF

For wheat-reared WBF,

$$\begin{array}{lcl} \text{Mean no. flies/plant} & = & 1.30 + 0.06 (\text{Plant species}) - 0.19 (\text{Plant height rank}) \\ \text{Standard Error} & & \begin{array}{cc} 0.20 & 0.24 \\ & 0.05 \end{array} \end{array}$$

The relationship with plant height rank is very highly significant ($P < 0.001$), but the relationship with plant species is not significant at all ($P = 0.81$). This implies that wheat-reared WBF chose couch plants to rest upon, on the basis of plant height rather than plant species.

6.2.3. Conclusions and discussion

Whether WBF are raised on couch or wheat, the adult flies are attracted more to couch plants than to wheat plants. However, couch-raised flies make this choice on the basis of plant species, whereas wheat-raised flies make the choice on the basis of plant height. This has very interesting implications for the ecology of the fly and for its control. It may be that, during their development, couch-raised WBF are conditioned to attractants in the host species, whereas those raised on wheat are not.

This experiment was not designed to investigate adult WBF choice for plants of different heights. When the experiment started in early May, the plants were all of a similar height, but in the following 8 weeks, the couch plants grew more vigorously than the wheat plants, despite addition of 0.4g ammonium nitrate fertiliser to the wheat plants on 9th June. At the start of the experiment, the plants were probably less mature than those which adult flies might encounter in the field when they emerge in late June or early July. Plant height could be easily controlled by growing many more couch and wheat plants, and carefully selecting for bioassays plants of the same height. Jones and Moore (1978) had solid roofs on their WBF rearing cages, whereas in the present study the roofs were of nylon mesh. Solid roofs might have reduced any possible confounding effect from the attractiveness of light to the flies. Shaded cage roofs and mature plants as tall as the cage might have produced clearer results.

6.3. 1999 field trial on adult wheat bulb fly plant choice

6.3.1. Introduction

McAlpine and Slight (1981) found WBF adults in Canada almost always associated with couch grass, although previous workers in Britain (e.g. Gough, 1946) had found plenty in wheat fields. The present study compared numbers of WBF adults in spring and winter wheat, along a hedgerow where the dominant grass was couch, and along an adjacent fence where couch also dominated.

6.3.2. Materials and methods

The experimental site, shown in Fig. 6.5 below, was a wheat field on Sydserf Farm, North Berwick, East Lothian, bounded on the north by a rough track and a blackthorn hedge or fence (OS reference NT547821). On 5th August 1999, REBELL yellow sticky traps (Swiss Federal Research Station for Fruit Growing, Viticulture and Horticulture, Wädenswil) were fastened on bamboo poles 80cm above the soil surface, and five each were placed in 3 separate locations:

- at 10m intervals along the hedge
- in a parallel line in the centre of a 128m wide band of spring wheat
- in a further parallel line 59m into an adjacent band of winter wheat.

The following week, another treatment was added, further east along the field boundary, where the hedge was replaced by a wire fence.

The wheat was harvested during the week following 23rd August; both wheat treatments were discontinued after this date.

At weekly intervals, the sticky traps were removed and replaced, and numbers of WBF adults on each trap were recorded.

Fig. 6.5. Field site, adult WBF plant preference trial, Aug 1999

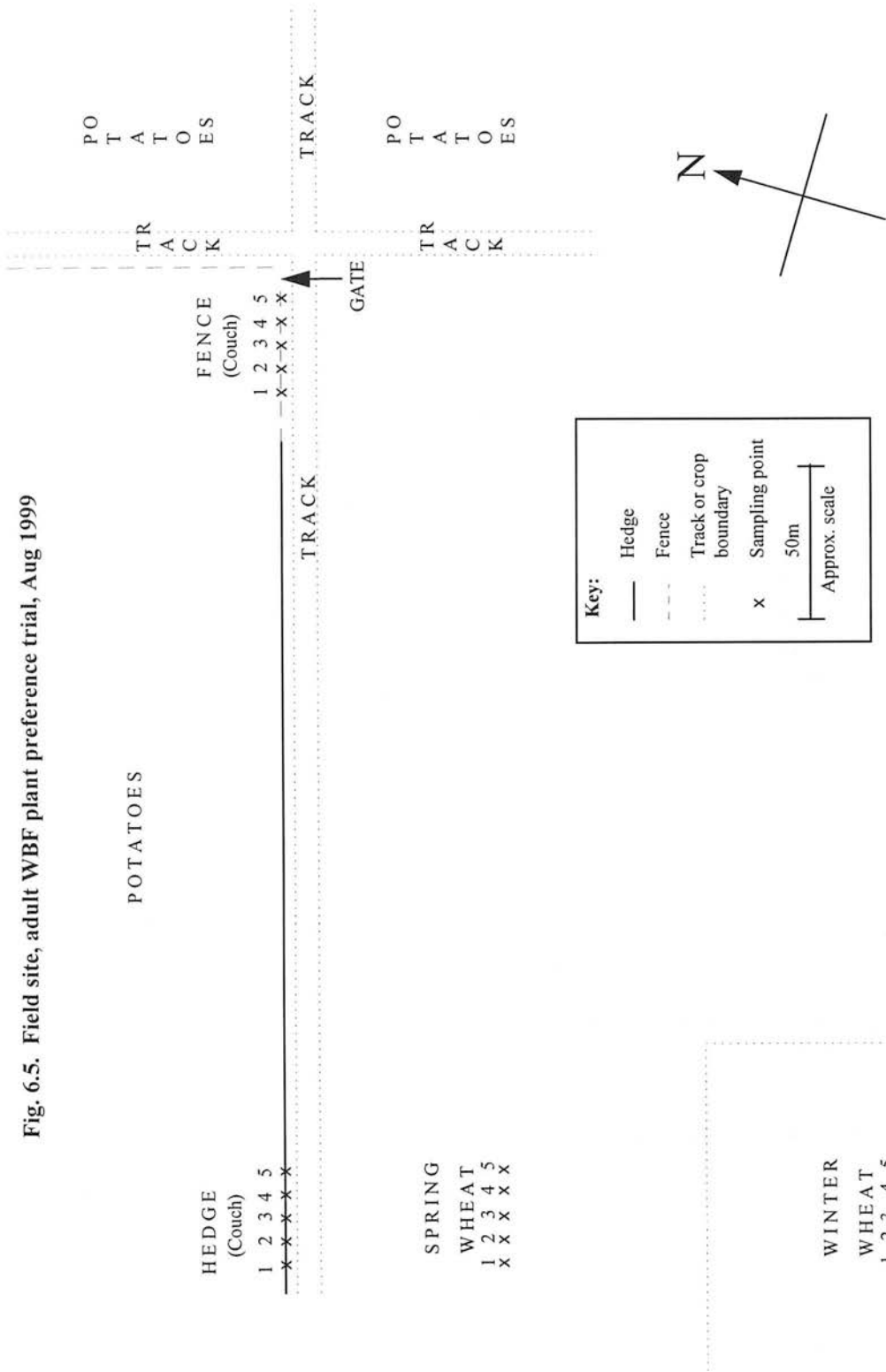


Fig. 6.5. Field site, adult WBF plant choice trial, August 1999

6.3.3 Results and discussion

Results are shown in Fig. 6.6 and Table 6.4, below:

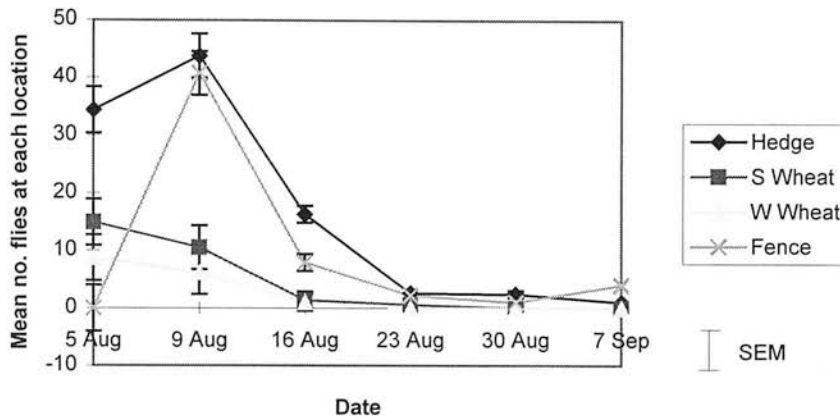


Fig. 6.6. Field plant choice of adult WBF - 1999

Table 6.4. Field plant choice of adult WBF - 1999

Mean no. flies at each location in week following:						
Location	5 Aug	9 Aug	16 Aug	23 Aug	30 Aug	7 Sep
Hedge	34.4 a	43.8 a	16.4 a	2.6	2.4 a	1.0
S Wheat	15.0 b	10.6 b	1.4 c	0.6	N/A	N/A
W Wheat	8.8 b	6.2 b	1.0 c	0	N/A	N/A
Fence	N/A	40.8 a	8.0 b	2.2	1.0 b	4.0
Significance	P<0.01	P<0.001	P<0.001	NS	P<0.05	NS
SEM	4.00	3.81	1.48		0.42	

Analysis of variance showed that:

- In the first week there were significantly more flies found in the hedge than at any other location
- In week 2, there were significantly more flies found in the hedge and under the fence than in either wheat location.
- In week 3, there were significantly fewer flies in the wheat, and significantly more in the hedge than under the fence.
- In week 5, there were significantly more flies in the hedge than under the fence.

Gough (1946), in a similar experiment, found that “unless the boards were examined within two days the flies could not be identified with any certainty”, and in the present case, too, identification of dried and blackened flies was difficult. In some cases the sticky traps had fallen to the ground when they were collected, but the number of WBF on them was still recorded. After the third week, the numbers of flies were very low, which suggests that the trial missed the early stages, and possibly the peak, of WBF emergence. Thus the only reliable results are those from week 3. These support the hypothesis that adult WBF prefer couch to wheat, but it was decided to repeat the trial the following year, starting in late June, and using improved methods to assess fly numbers. It was felt that a non-destructive method of sampling would be preferable.

6.4. 2000 field trial on adult wheat bulb fly plant choice

6.4.1. Introduction

Following the inconclusive results of the 1999 field trial, next year the trial was repeated in the neighbouring field to the north at Sydserf Farm, starting earlier in the season and using improved, non-destructive methods.

6.4.2. Materials and methods

The trial was undertaken in the south-east corner of the field directly behind Sydserf Farm steading, shown in Fig. 6.7, overleaf. The field had been sown with winter wheat, and treated conventionally throughout the season. Five posts 10m apart along the same length of fence used in the 1999 trial were marked on the southern edge of the field, starting 7m from the south-eastern corner. Parallel to these marked posts, five marked bamboo poles were placed 35m into the crop. Each week from 29th June to 24th August, four sweeps were made of the plant tops with a 35cm diameter x 60cm deep sweep net at each location. Adult WBF were removed from the net with an aspirator, identified, sexed and counted, and then released.

Gough (1946) found more adults at the edges of a wheat field, and especially at an open edge bordering root crops, than in the centre of the field. To check on the influence of the crop edge, and of the orientation of the line of sampling points, 40m sweeps were made of the two lines of sampling points, of a section of the north-south fence where couch also predominated, and of the parallel edge of the wheat crop in the next field, which bordered a dirt track. Once again, adult WBF were removed from the net with an aspirator, identified, sexed and counted, and then released

The crop was harvested in the week following 24th August.

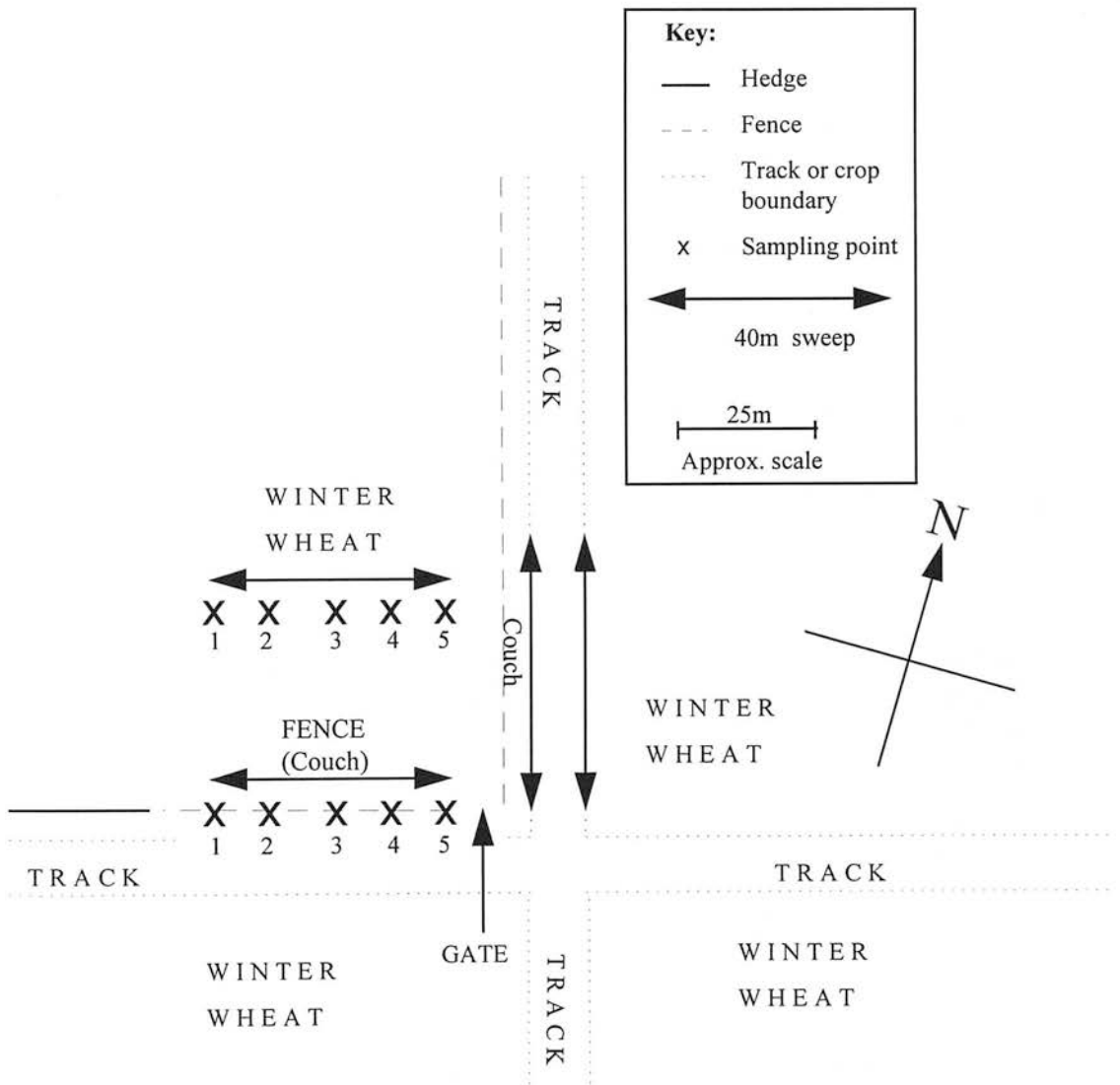


Fig. 6.7. Field site, adult WBF plant choice trial, July – August 2000

6.4.3. Results

The mean numbers of adult flies of both sexes found at each sampling point are shown in Fig. 6.8 below:

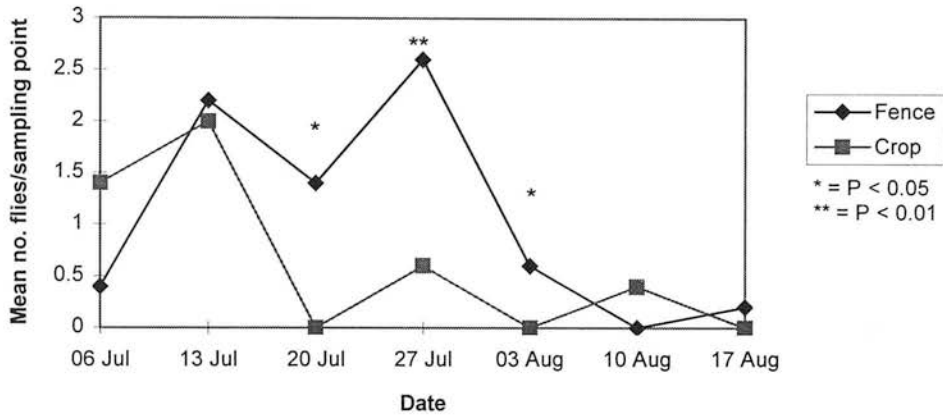


Fig. 6.8. Field plant choice of adult WBF – 2000

Between 20 July and 3 August, there were significantly more flies found at the fence than in the crop. This implies that the flies preferred couch grass to wheat. There were no flies found before 6 July or after 17 August.

When male and female flies are considered separately, significantly more males were found on couch than on wheat on the 27 July, and significantly more females were found on couch than on wheat on 3 August (Fig. 6.9., below)

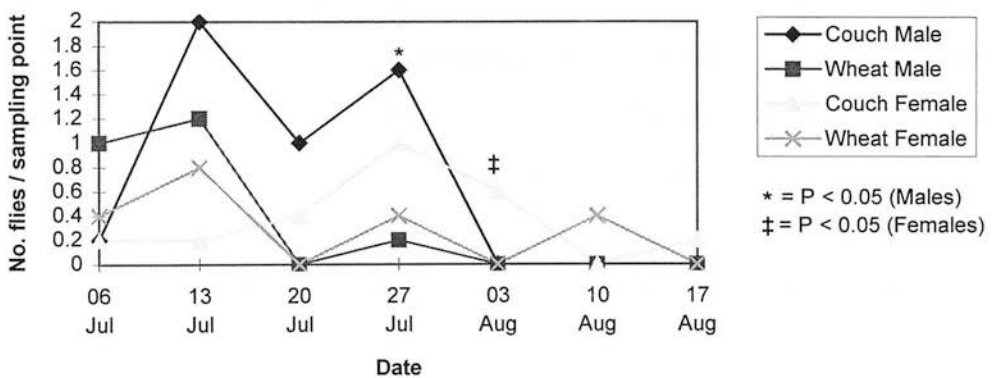


Fig. 6.9. Field plant choice of adult WBF by sex – 2000

However, when the 40m sweeps are taken into account, the results are less clear. Throughout the experiment, there were no significant differences between the

number of flies found over couch and over wheat, or between the number of flies found in north-south and in east-west sweeps (Fig. 6.10). However, this may be due to there being only two replicates in each treatment. The numbers of flies found within the wheat crop were consistently low, although the numbers of flies found in other sweeps varied greatly.

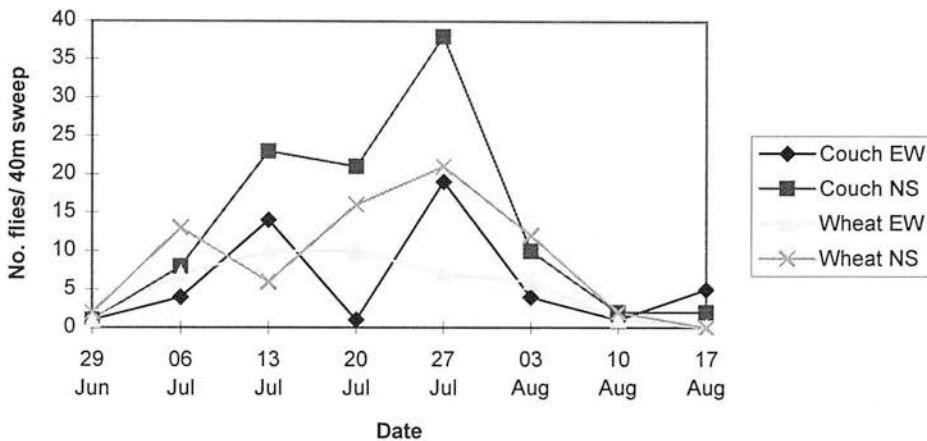


Fig. 6.10. Field plant choice of adult WBF
40m sweeps, 2000

6.4.4 Conclusions from 2000 adult plant choice field trials

From 20 July to 3 August, significantly more adult WBF were found on couch plants under a wire fence than on wheat plants in a standing crop. This may indicate a preference for couch plants over wheat plants. However, 40m sweeps, although not producing significant results, found consistently low numbers of WBF within the wheat crop. This may support Gough's (1946) observation that higher numbers of WBF adults were found at the open edge of a crop of wheat.

If sampling points along the two north-south 40m sweeps, either side of a dirt track, had been used, the results might have been clearer. It should also be noted that, although couch was the dominant grass under the wire fences, other grasses, and cow parsley (*Anthriscus sylvestris* L.), were also present. A pure stand of couch grass would be hard to find, and deliberate sowing or planting of this pernicious weed would be unacceptable to the owners or farmers of a field site.

6.5. Discussion

Laboratory experiments showed that adult WBF preferred resting on couch to wheat, although in the case of wheat-reared flies, this choice was based on plant height rather than on host plant species. In the field, the flies appeared to prefer couch to wheat, although because of the trial design, a preference for the edge rather than the centre of the crop, as suggested by Gough (1946), cannot be ruled out.

These findings appear to demonstrate Hopkins' host-selection principle (Hopkins, 1917) that insect larval experience of host plant could affect adult host plant and oviposition preference. However, this has yet to be conclusively demonstrated, and, based on a wide range of previous authors' findings (Szentesi & Jermy, 1990), it is much more likely that this is an example of early adult, rather than larval, conditioning, as demonstrated by Barron and Corbet (1999) in *Drosophila melanogaster*. To test this hypothesis, it would be necessary to remove all traces of the larval host from pupae, as Barron and Corbet (1999) did, before eclosion and bioassays of adult flies.

In future research the two effects of plant height and host plant species should be separately studied, ideally with a turntable apparatus, as used by Hopkins (1994). This could be used to test either WBF response to couch and wheat plants of a standard height, or plants of varying height of one species or the other. To preclude any olfactory effects, dummy plants similar to those used by Hopkins (1994) could be used instead of real plants to test WBF response to plant height.

The requirements of the flies must be; first, an adequate supply of food (mostly water, honeydew and saprophytic organisms on the plant surface (Jones, 1970b)); second, shelter from extremes of weather and temperature; and later, members of the opposite sex for mating. A preference for taller grasses might serve to bring mating pairs together, as might a preference for grasses at the edge of a stand or crop, especially in a species such as couch with relatively few flowering heads (Palmer &

Sagar, 1963). However, the latter preference might also be an effect of flies returning from foraging trips, or of aggregation on the lee side of the crop, as suggested by Long (1958d), or female flies returning from laying eggs in nearby bare soil. Very few aphids were seen in the present study, and their presence or absence was not recorded in previous studies. The “edge effect” needs further investigation, and it would be interesting to note whether it occurs in wheat crops with heavy aphid infestations and hence substantial supplies of honeydew; if not, this would suggest that, prior to oviposition, adult WBF need only leave the emergence site to forage for food.

Bardner, Jones and Coaker (1969) found from electro-antennograms that female WBF could detect damp, dead wheat leaves, or living foliage. This would suggest that choice of feeding and resting sites may be based on olfactory cues as well as visual cues such as plant height or proximity to the edge of the crop.

Prokopy (1968) found that the visual response of the tephritid *Rhagoletis pomonella* (Walsh) depended on the size, shape and colour of visual cues. Roessingh and Städler (1990) showed that gravid cabbage root flies selected artificial leaves with a stem, instead of those without, for oviposition.

The present study and previous research suggest that the choice of resting site in adult WBF depends on a complex interaction of responses to visual and olfactory cues, and a complex interaction of requirements for food, shelter and mating. Only further research can unravel these interactions. The interaction of visual and olfactory cues could be tested with plants behind glass or mesh screens in a wind tunnel, as used by Hitimana (2000).

Chapter 7

Choice of oviposition sites by female wheat bulb flies

Chapter 7. Choice of oviposition sites by female wheat bulb flies

7.1. Introduction

Ever since the wheat bulb fly (WBF) was first reported as a pest of wheat (Ormerod, 1883) it has been observed that attacks were worst on wheat following fallow, and by 1920 it was well-established that “the worst attacks follow a bare fallow or bastard fallow and that bad attacks also occur after crops of potatoes, rape, swedes, turnips and mangold, especially where the soil is bare during the summer” (Petherbridge, 1921). To this list of crops may be added open canopy vegetable crops such as onions, or low standing crops such as peas or sugar beet (Young & Ellis, 1996). No evidence has ever been found of attraction to these crops themselves, and it appears that the gravid female prefers to lay her eggs in bare soil, even if there is no sign of a suitable host plant.

However, if WBF larvae prefer couch to wheat (Chapter 2), and since there is some evidence of an adult preference for couch over wheat (Chapter 6), it could be that gravid female WBF choose to lay their eggs in apparently bare soil over buried couch rhizomes, rather than in truly bare soil. This hypothesis was tested in the laboratory and the field.

7.2. Choice of oviposition site by female wheat bulb flies in the laboratory

7.2.1 Materials and methods

Adult WBF emerging from the crates of wheat or couch described in Chapter 5 were transferred to two separate cages depending on their larval host plant. The cages were identical to those described in Chapter 6, and were kept in the same growth room, but the roofs were covered in cardboard to provide shade. Each contained one plant of the same species on which the larvae had been reared, in a 20cm pot of compost, standing in a dish of water. On top of each pot was a perspex shelf in two

halves which fitted around the base of the plant. On the shelf were four 4cm diameter Petri dishes containing food as described in Chapter 6. The food was replaced twice a week, and on intervening days 5ml of sterile distilled water was added to each dish. Every day the plants and the sides and roof of the cage were thoroughly soaked with a fine spray of water, and any gravid female flies were transferred to separate oviposition cages, depending again on the plant used for larval rearing.

The oviposition cages were very similar to those used by Bardner and Kenten (1957), and in the present study in 1999, which had been shown to encourage oviposition. The cages, (Fig. 7.1, overleaf), were clear plastic cylinders, 17.5cm diameter x 30cm high. The tops and bottoms of the cages were metal with a central 10cm diameter hole covered with 1mm nylon mesh. On top of the upper mesh, 4 dental wicks soaked in food as detailed above were held upright in plastic fluorimetric cuvettes (Hughes and Hughes Ltd, Romford, Essex) supported upside down in a section of the plastic tray in which they had been packed. The wicks and cuvettes were replaced twice a week, when the nylon meshes were cleaned. On intervening days the wicks were soaked with sterile distilled water. Under the lower mesh were 4 plastic trays made from a quartered 14cm diameter Petri dish and filled with horticultural grit sand saturated with sterile distilled water. In two trays, 6cm sections of couch rhizomes were buried under the sand along the radius of the dish; the other, control, trays contained no rhizomes. The two treatments were placed in alternate positions.

Every day the trays were checked for WBF eggs, and if any were seen, they were removed by washing and sieving, and then counted, after which fresh trays were prepared. To control for any effects from the relative positions of food and sand trays, the cuvettes were moved clockwise, and the trays anti-clockwise, every day. As in the adult plant choice bioassays, better control for factors such as tray position and lighting could have been achieved with a turntable apparatus as used by Hopkins (1994) for testing the host plant preferences of cabbage root fly, but the numbers of flies raised for the present study would not permit this.

On 28 May, shortly after the first couch-reared gravid females were transferred to an oviposition cage, most of the adult flies died as a result of over-heating in the growth room. Thereafter, the oviposition cages were kept in a Fison's cabinet under the same climatic conditions as the growth room. Thus the oviposition bioassay was conducted only on wheat-reared females, which emerged later, and it was not possible to compare fecundity of couch-reared and wheat-reared WBF.

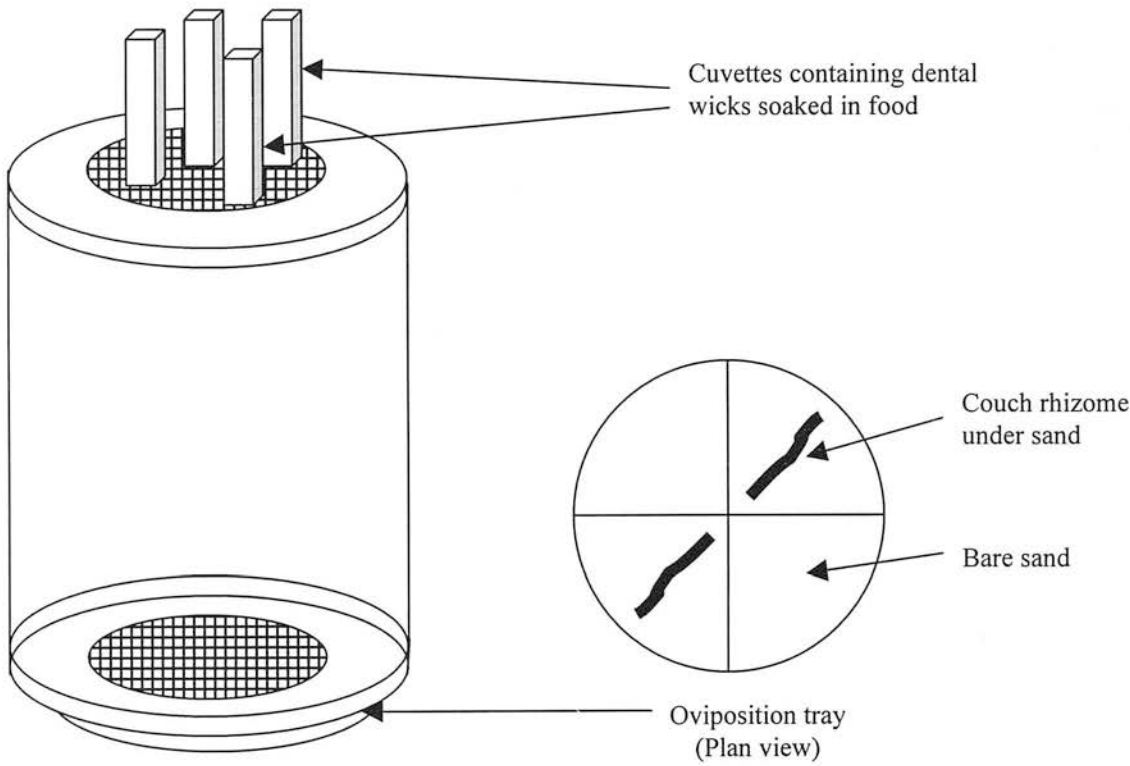


Fig. 7.1. WBF oviposition choice test bioassay

7.2.2. Results

Each of the 13 days when WBF eggs were found and counted was treated as a replicate. Over these 13 days, there was no significant difference between the mean number of eggs laid in bare soil and over couch rhizomes (Table 7.1).

Table 7.1. Oviposition site choice by female WBF (laboratory)

Mean no. eggs laid / female, \pm SE, in:	
Bare grit sand	2.46 ± 0.64
Grit sand over couch rhizomes	1.28 ± 0.64

7.2.3. Conclusions of laboratory experiment

Under these experimental conditions, gravid female WBF were not encouraged to lay their eggs by the presence of buried couch rhizomes. It is, however, possible that the flies were unable to discriminate between the two treatments when they were so close together.

7.3. Choice of oviposition site by female wheat bulb flies in the field

7.3.1. Introduction

Gough (1946) found that, on light soils, WBF damage was most common after potatoes, and McKinlay and Franklin (1980) found that, in Scotland, the highest numbers of WBF eggs were found at the tops of potato ridges.

Cooper (1978) found that, in the Lothians, the numbers of gravid females at oviposition sites peaked at the end of July and early August, with a second peak in late August in only two of the four years under study.

It was thus decided to site the current field trial in East Lothian, between the tops of potato ridges, from late July to early September.

7.3.2. Materials and methods

The experimental site was a seed potato field adjacent to a wheat field on Newhouse Farm, East Lothian, where large numbers of WBF eggs can be found every year (OS reference NT 538831). The potatoes (cv. Maris Piper) were conventionally sown and treated; full details are given in Appendix 3. Ten large plastic seed trays 38 x 57 x 8cm deep, with holes in the base for drainage, were filled with horticultural grit sand. In five trays, two lengths of couch rhizomes were buried across the diagonals. On 6th July 2000, control and treatment trays were laid lengthwise in pairs (i.e. five replicates) 30cm apart between the potato ridges, spanning a furrow three rows south of the neighbouring wheat. Within each pair, treatments were randomly allocated. The distance between each pair of trays was 10m. At weekly intervals, any WBF eggs were removed from the tray contents by sieving and flotation, as described in SAC Standard Operating Procedure CER 020 (Appendix 1) and counted. At the same time, the trays were replaced with fresh ones.

7.3.3. Results

Results are shown in Fig. 7.2 and Table 7.2. There was no significant difference between the number of eggs laid in either treatment on any date, nor in total. The peak in egg-laying occurred in the week preceding 17 August.

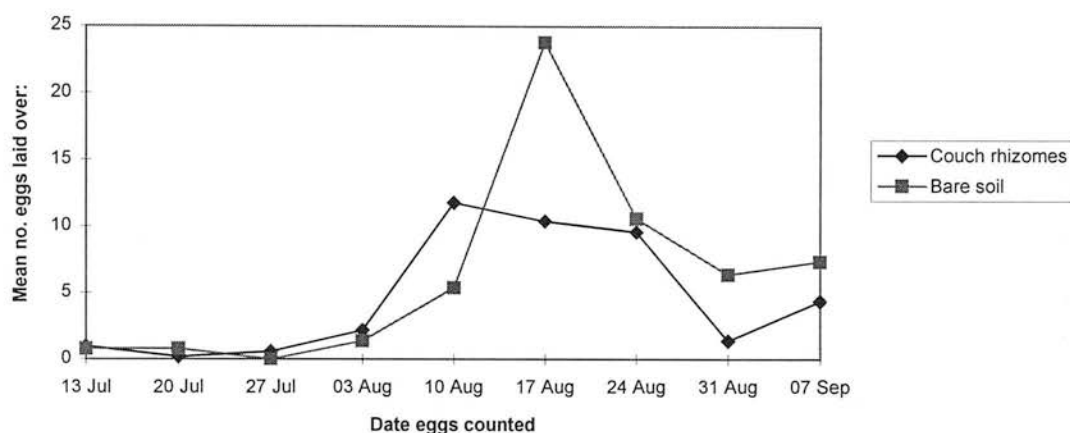


Fig. 7.2. Choice of field oviposition sites by female WBF, 2000

Table 7.2. Choice of field oviposition sites by female WBF, 2000

	Mean no. eggs laid over:									
Week preceding	13 Jul	20 Jul	27 Jul	03 Aug	10 Aug	17 Aug	24 Aug	31 Aug	7 Sep	Total
Couch rhizomes	1	0.2	0.6	2.2	11.8	10.4	9.6	1.4	4.4	41.6
Bare soil	0.8	0.8	0	1.4	5.4	23.8	10.6	6.4	7.4	56.6

7.5. Conclusions and discussion

Buried couch rhizomes did not encourage gravid female WBF to lay eggs, either in the laboratory or the field. Bardner *et al.* (1969) found from electro-antennograms that female WBF could detect damp, dead wheat leaves, or living foliage, but there is no evidence that gravid females are attracted to any of the crop plants under which they are known to lay eggs. The attractiveness or otherwise of couch plants or any other wild grasses as oviposition sites has never been investigated, but neither has it been observed, so this remains a possibility. Indeed, this may explain the case reported by Shaw and White (1969) of WBF damaging a crop of spring barley sown

in May, in areas of the field known to be heavily infested with couch. Palmer (1958) found that couch rhizomes grew horizontally during the spring and summer before forming a primary aerial shoot in the autumn, and that plants in open communities produced secondary tillers and rhizomes during their first growing season. Both these factors might favour gravid WBF laying eggs on or near isolated primary aerial shoots growing from rhizomes in the autumn.

Even if the flies are attracted only to bare soil, and not to any plants, for oviposition, this does not rule out couch as the principal host. Couch rhizomes are very vigorous colonisers of bare soil, and so couch shoots are very likely to be found in bare soil near an existing couch plant. Furthermore, laying eggs in bare soil near the host plant, rather than right beside it, might increase the chances of WBF larvae finding young couch shoots susceptible to invasion, rather than older shoots which are too tough. Bardner and Fletcher (1973) found fewer WBF eggs and larvae near trees and hedges than in the centre of the field, to a distance roughly equal to the height of the vegetation. They suggested this was due to negative hypsotaxis of egg-laying females. This would imply that gravid females resting on couch or other grasses at the edge of a stand (see Chapter 6), once their eggs have matured, might seek out bare soil slightly further away than the height of their resting sites. Such oviposition sites would be highly susceptible to invasion from new couch rhizomes and shoots in the following months.

Bardner *et al.* (1969) found from electro-antennograms that female WBF did not respond to the odour of soil. Griffiths and Scott (1968), however, found that flies laid twice as many eggs on filter paper treated with an aqueous extract from peat/sand compost as with water, but showed no preference for an extract of soil. They also found that flies laid more eggs on black than on white surfaces, and when offered an alternative colour to black laid more eggs on brown and green surfaces (Griffiths & Scott, 1968). Raw (1955), and Sol (1971) showed that WBF laid more eggs on soil with a rough texture. Sol (1971) found that WBF preferred to lay their eggs in dry soil, and this was confirmed by von Grafenstein and Ulber (1989). Von

Grafenstein and Ulber (1989) attributed this and the flies' preference for laying eggs in soil of small particle size to the mobility of small, dry soil particles, since the females tried to insert their ovipositor as deep as possible into the soil. Oviposition dishes containing coarse sand glued to the base of the dish were rarely accepted by the females, probably because they were not able to move the particles. In related field experiments Kaack, Ulber and von Grafenstein (1989) concluded that female WBF's preference for laying eggs in coarse, clodded fallow, or under crops with a high leaf area index such as potatoes and sugar beet was due to the shaded, cool, and humid microhabitats in both situations.

One can thus hypothesise a catenary process by which gravid WBF find suitable oviposition sites. Gravid females are probably deterred from laying eggs within tall crops such as cereals, even when thinned, because they rarely descend more than 46cm into a crop (Long, 1959). On leaving the crop or stand of grass where they have been resting and feeding, once beyond its hypsotactic shadow, they are visually attracted to the brown colour of soil, or the green and brown colour of a broad-leaved crop with bare soil beneath. Thereafter, the female might test potential oviposition sites for suitable moisture content and penetrability of the soil with her ovipositor, and move on to another potential site if the first proved unsuitable.

Chapter 8

Summary, conclusions and discussion

Chapter 8. Summary, conclusions and discussion

8.1. Summary of results and conclusions

8.1.1. Choice test bioassays of neonate wheat bulb fly larvae

Wheat bulb fly (WBF) neonate larvae:

- are positively geotactic
- are negatively phototactic
- are more attracted to couch seedlings and their exudates than to alginate gel controls
- are more attracted to couch rhizome exudates than to alginate gel controls
- are more attracted to couch seedlings and their exudates than to wheat seedlings and their exudates

Both couch and wheat seedling exudates are more attractive than aggregated alginate gel controls. However, when directly compared to control gels, only couch seedling exudate is more attractive. When couch and wheat seedling exudates are directly compared the couch exudate is less attractive than when compared to controls; this implies that the larvae are confused by two sources of attractants, even when one source is stronger than the other.

Wheat exudate is more arrestant than couch seedling exudate or controls. However, alginate gel controls also have arrestant properties.

No chemical analysis of wheat or couch exudates was undertaken in the present study. However, previous studies, notably Copaja *et al.* (1991), and Niemeyer *et al.* (1992), have found higher levels of the hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in WBF's two most favoured host plants, couch and wheat, than in less favoured or rejected species. DIMBOA thus merits further research as a possible attractant or arrestant of WBF larvae.

8.1.2. Interaction with host plants during larval development

Couch and wheat respond differently to attack by WBF larvae. Both produce extra shoots in the early infestation, although this response is more marked and more rapid in couch than in wheat. However, these extra shoots are soon consumed by the developing larvae, and after 5 weeks, infested plants of both species have fewer shoots than clean plants. WBF larvae are more likely to kill wheat plants than couch plants, but clean wheat plants are better able than clean couch plants to compensate for the death of neighbouring infested plants. Thus compensation for WBF attack occurs both within plants, as reported by Long and Morris (1961), and between plants, as reported by Bardner *et al.* (1969). Within-plant compensation is more marked in couch than in wheat, probably because of the extra reserves for shoot production available in couch rhizomes, and inter-plant compensation is more marked in wheat than in couch. However, within-plant compensation for WBF infestation is not complete in either species, as shown by the reduced number of leaves per infested plant, and hence, reduced photosynthetic potential.

8.1.3. Survival and development rate of WBF larvae and pupae

WBF adults emerge earlier when reared on couch than when reared on wheat. This accords with Gemmill's statement (1927) that "a newly-hatched larva can complete its life-history, up to the emergence of the fly from the pupa, rather more quickly (8 weeks) even than in wheat (8½ to 9 weeks)". This would account for their preference for couch over wheat as larvae (See Chapter 3), and may be due to their higher consumption of couch shoots than wheat shoots in the early stages of infestation (See Chapter 4). Earlier eclosion, even by a few days, would allow more time for mating and egg-laying.

The more rapid development of larvae and pupae from older eggs has not been reported before, and was entirely unexpected. One can hypothesise that earlier-laid eggs would develop more fully before diapause, and would need less time to develop as larvae. If this hypothesis could be proven in further research, by artificially

varying the length of pre-diapause and diapause, it would add further weight to the importance of early eclosion and hence earlier egg-laying. In order to be certain about the date of egg-laying, such research would have to rely on laboratory-reared eggs, rather than eggs collected from the field.

8.1.4. Plant choice in adult wheat bulb flies

Laboratory-reared adult flies are attracted more to couch plants than to wheat plants. However, couch-reared flies make this choice on the basis of plant species, whereas wheat-reared flies make the choice on the basis of plant height. This has very interesting implications for the ecology of the fly and for its control. It may be that, during their larval development, couch-raised WBF are conditioned to attractants in the host species, whereas those raised on wheat are not.

In the field, the flies appeared to prefer couch to wheat, although because of the trial design, a preference for the edge rather than the centre of the crop, as suggested by Gough (1946), cannot be ruled out.

8.1.5. Choice of oviposition sites by female wheat bulb flies

Buried couch rhizomes did not encourage gravid female WBF to lay eggs, either in the laboratory or the field. There is no evidence that gravid females are attracted to any of the crop plants under which they are known to lay eggs. The attractiveness or otherwise of couch plants or any other wild grasses as oviposition sites has never been investigated, but neither has it been observed, so this remains a possibility. Indeed, this may explain the case reported by Shaw and White (1969) of WBF damaging a crop of barley sown in May, in areas of the field known to be heavily infested with couch. Palmer (1958) found that couch rhizomes grew horizontally during the spring and summer before forming a primary aerial shoot in the autumn, and that plants in open communities produced secondary tillers and rhizomes during their first growing season. Both these factors might favour gravid WBF laying eggs on or near isolated primary aerial shoots growing from rhizomes in the autumn.

However, even if the flies are attracted only to bare soil, and not to plants, for oviposition, this does not rule out couch as the principal host. Couch is a very vigorous coloniser of bare soil, and so very likely to be found in bare soil near an existing couch plant. Furthermore, laying eggs in bare soil near the host plant, rather than right beside it, might increase the chance of WBF larvae finding young couch shoots susceptible to invasion, rather than older shoots which are too tough. Bardner and Fletcher (1973) found fewer WBF eggs and larvae near trees and hedges than in the centre of the field, to a distance roughly equal to the height of the vegetation. They attributed this to negative hypsotaxis of egg-laying females. This would imply that gravid females resting on couch or other grasses at the edge of a stand (see Chapter 6), once their eggs mature, might seek out bare soil slightly further away than the height of their resting sites. Such oviposition sites would be very susceptible to invasion from new couch rhizomes and shoots in the following months.

8.2. Discussion; the ecology and evolution of wheat bulb fly

These research findings establish that WBF larvae are attracted more to couch than to wheat. They develop faster on couch than on wheat, possibly because couch responds to WBF attack with extra shoot production sooner and more vigorously than wheat. WBF adults are more likely to rest on couch than on wheat, and on taller plants. There is some suggestion that adults are also drawn to plants on the edge of stands. Buried couch rhizomes do not encourage gravid WBF to lay eggs, but their response to young couch shoots has never been observed or tested. These findings, and those of previous behavioural studies (Raw & Stokes, 1958; Scott, 1974), and the comparisons of WBF's geographical distribution and phenology with those of its hosts in Chapter 1, suggest that couch is indeed the natural host of wheat bulb fly. Raw and Stokes (1958) found more WBF larvae in couch than in any other test plant, including wheat, and Scott (1974), while not testing couch, found that none of his test grasses gave a higher "arrestancy value" than that of wheat.

If couch is the natural host of WBF, and gravid females do indeed lay their eggs in bare soil, what can be inferred about the ecology and evolution of the insect?

WBF larvae only attacks young shoots (Long, 1960a), so those infesting couch must have hatched from eggs laid mainly in bare soil being invaded by couch seedlings, or by rhizomes from established couch plants. This implies a patchy habitat.

It is clear that, in order to understand the ecology of WBF and its hosts, relationships between host, insect and their environment need to be considered on several different spatial and temporal scales. The increasing spatial scales suggested by Hassell and Southwood (1978), of plant unit, patch, and habitat seem appropriate. However, it should be noted that the definition of patches in the case of WBF and couch would be unconventional. Unlike the example illustrated by Hassell and Southwood, the centres of couch clumps would be unsuitable, surrounded by a ring of suitable food units, and interspersed by bare soil of uncertain suitability (Fig. 8.1).

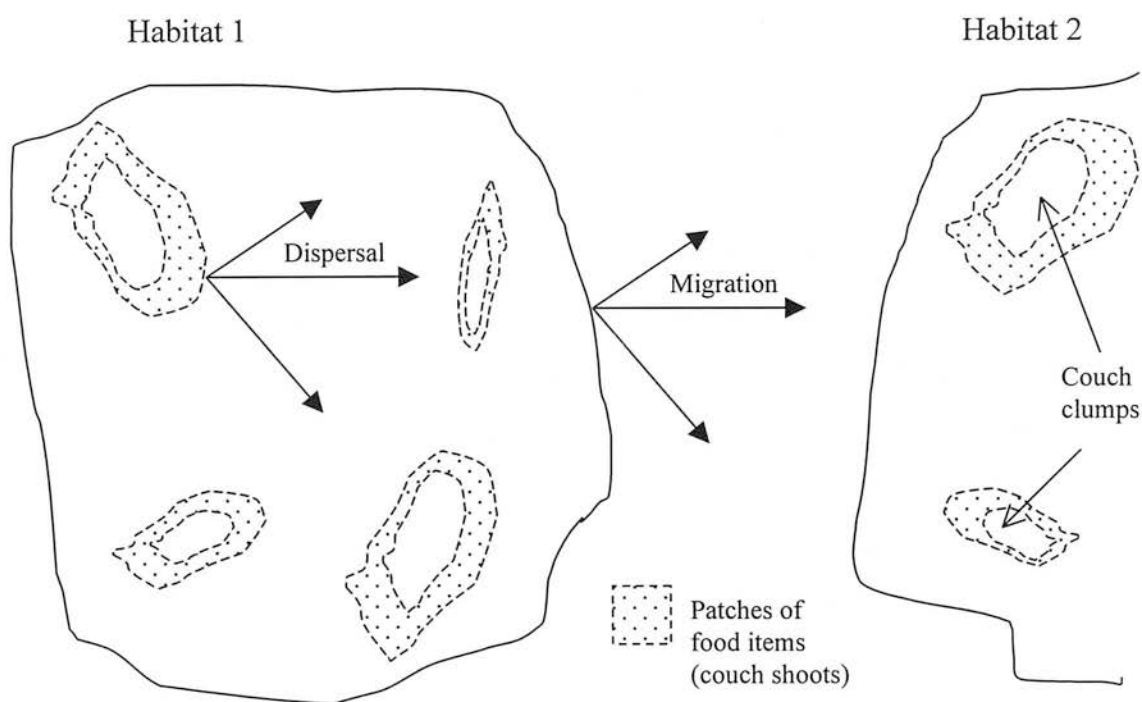


Fig. 8.1. Diagram illustrating the divisions of habitat, patch and food item. Bold arrows indicate migratory movements between habitats and dispersal between patches. After Hassell and Southwood (1978).

Similarly, one can consider increasing temporal scales of one WBF generation (conveniently, one calendar year), the lifespan of an appropriate habitat, and evolutionary time.

8.2.1. Reconstruction of WBF life cycle on couch grass

It is possible to reconstruct a tentative life cycle of WBF on couch, i.e. the relationship between insect and host on the smallest spatial scale of individual plant units and the shortest temporal scale of one insect generation.

On eclosion in late June, adult WBF are attracted to the tallest grasses, especially couch, towards the edge of the stand of grass (Gough, 1946). There they feed on the saprophytic micro-organisms on flowering couch ears (Jones, 1970b), produced in May and June (Palmer, 1958). The fact that only a few couch shoots produce ears (Palmer, 1958) may assist the aggregation of WBF adults for mating.

After mating, gravid females remain at feeding sites for 5 weeks until their eggs have matured (Jones 1970b), before finding a suitable oviposition site. Legowski, Maskell and Williams (1968), and Bardner, Lofty and Huston (1968), suggest that the flies disperse no further than 0.8km, and mostly downwind, which implies that they would lay eggs in the first suitable site they encounter on flying outwards from the edge of the grass stand. Bardner and Fletcher (1973) found that “eggs and larvae of WBF were fewest near trees and hedges for a distance approximately equal to the height of the vegetation, probably because egg-laying flies are negatively hypsotactic and avoid the vicinity of prominent objects on the skyline”. Interestingly, this accords with one of the earliest observations on WBF by a farmer, reported by Ormerod (1883), that the insect “always leaves a belt of five or six yards near the hedge untouched”. Couch may grow up to 1.2m high, so negative hypsotaxis would discourage egg-laying within 1.2m of the edge of the plant stand.

Palmer (1958) found that couch growing in open communities (as on the edge of a plant stand) would continue horizontal rhizomatous growth for up to 2m, then

produce terminal aerial shoots between August and October, when WBF females are still laying eggs. Marshall (1990) found that bare plots encouraged rhizome growth of couch by a factor of 10 compared with plots sown with six perennial grasses. Two hypotheses emerge as to how the egg-laying behaviour of adult WBF might favour neonate larvae seeking suitable couch shoots the following January. The first hypothesis is that young couch shoots, as found in late summer, encourage gravid WBF to lay eggs nearby. The response of gravid females to young couch shoots has never been observed or tested, although Shaw and White (1969) suggest that “even though reasonable cover combined with a couch infestation would suggest an unsuitable oviposition site for wheat bulb fly, a substantial amount of a wild host is probably a sufficient stimulus to initiate egg laying.”

Even if this is not the case, and gravid females respond only to bare soil as a stimulus to egg-laying, the first bare soil they encounter from which they are not discouraged by negative hypsotaxis would be in the area where couch rhizomes would produce aerial shoots in the autumn and very early spring (second hypothesis). Klein and van Groenendael (1999) found that, in unproductive soil, couch rhizomes selectively invaded bare soil rather than soil in supporting other plants, and then produced more shoots; this would favour WBF larvae hatching from eggs laid in bare soil.

The first hypothesis could be easily tested by using couch shoots, rather than buried rhizomes, in bioassays and field trials like those in the present study. The second would be more difficult to test, but would probably require counting couch shoot numbers and WBF egg numbers in bands at progressively greater distances from clumps of couch in bare soil, and then analysing the relationship between these data.

Both hypotheses would result in eggs being laid in the area most likely to have many young aerial couch shoots when WBF larvae hatch in late January or early February. The present research shows that neonate larvae are more attracted to couch shoots than to wheat shoots, and thus, by extension, than to those of other hosts (Raw and Stokes 1958). Thus the high rates of mortality due to neonate larvae failing to find

wheat shoots as hosts may be much reduced in the more natural situation of the margins between an established stand of couch and bare soil being invaded by the couch. This is an area which has never been researched.

Laying eggs in bare soil may also greatly reduce the risk of predation. Ryan (1973a) showed in single-choice laboratory bioassays that WBF eggs were eaten by the carabid beetles *Agonum dorsale* Pont., *Trechus quadristriatus* Schr., and *Clivina fossor* L, and found more than 5 times as many carabids in the border of a wheat crop than in nearby fallow. In an earlier study (1967) he found that 50% of predation loss occurred between 17 August and 7 September, and 90% could be attributed to carabids. Jones (1975) found that 50-67% of eggs disappeared where known numbers were placed in the soil and controls were protected from predation. Most of the eggs in the latter study disappeared before the end of October, when *T. quadristriatus* was abundant. Lagerlöf and Wallin (1993) also found that carabids are more likely to be found in dense couch sod than in bare soil.

WBF larvae develop faster on couch than on wheat, possibly because couch responds to WBF attack with extra shoot production sooner and more vigorously than wheat. Even if a developing WBF larva were to kill all the apical shoots on a couch rhizome, the rhizome would respond by producing fresh shoots from the nearest node. Gemmill (1927) found that WBF larvae feeding within couch rhizomes could eat through the nodes. It is thus likely that such a larva would eat along the rhizome to the new shoots. Thigmotaxis in neonate WBF larvae can readily be observed in a Petri dish (pers. obs.); this too would assist larvae in finding new shoots.

WBF which have developed on couch emerge earlier as adults than those raised on wheat. Since females can survive for up to 75 days (Dobson and Morris, 1961), and their eggs take 5 weeks to mature (Jones, 1970a), earlier eclosion might just allow them enough time to mate twice, and lay a second brood of eggs before weather conditions become more adverse in the autumn. Cooper (1978) found some evidence for such behaviour in years with favourable weather conditions.

8.2.2. Effects of host plant on WBF mortality and population dynamics

How does a population of wheat bulb fly interact with couch on the larger spatial scale of a patchy environment?

There have been a number of studies on the mortality (cf. Raw, 1967; Ryan 1967, 1973a, 1973b, 1975) and the population dynamics (Bardner, Fletcher, Jones & Lofty, 1973; Kempton, Bardner, Fletcher, Jones & Maskell, 1974; Kowalski & Benson, 1978) of WBF. These in turn have formed part of several review papers (Dempster & Pollard 1981; Stiling, 1988; Hassell, Latto & May, 1989; Cornell & Hawkins, 1995; Ray & Hastings, 1996). Considering couch rather than wheat as the host plant of WBF could lead to a new interpretation of WBF population dynamics. Laying eggs in bare soil implies a patchy environment, and hence, a spatially diverse population density.

Ryan (1975b) found that the greatest sources of WBF larval mortality on wheat were failure to find a host plant, and starvation of third instar larvae which had killed their host plants. He suggested that both mortalities were density-dependent, since both were related to the number of shoots available to feed in (Raw, 1967; Ryan, 1975b). The life cycle of WBF on couch as outlined above would imply a reduction in both these mortality factors. Many neonate larvae would still fail to find a host, so this mortality factor would still be high. However, the present research has shown that couch responds more rapidly and vigorously than wheat to WBF infestation, and suggests that couch plants are less likely than wheat plants to be killed by WBF; thus starvation of third instar larvae may be significantly reduced when feeding on couch.

Cornell and Hawkins (1995) analysed the life tables of 124 holometabolous, herbivorous insects, including those drawn up for WBF by Bardner *et al.* (1973). They found significantly different survival patterns and mortality sources of insects depending on their lifestyle. The most important larval mortality source for endophytic insects, under which WBF was grouped, was natural enemies. This does

not agree with the principal larval mortality sources found by Ryan (1975b), which would be classified by Cornell and Hawkins (1995) as plant-derived (i.e. failure to find a host) for first instar larvae, and competition for third instar larvae. As explained above, it is likely that both these mortality sources would be less important in the more natural situation of WBF infesting couch.

Kowalski and Benson (1978), working from data covering 11 generations of WBF collected by Bardner *et al.* (1973), identified 5 different mortality factors as follows:

- k_1 - sterility or mortality of eggs in soil
- k_2 - mortality of 1st instar larvae when searching for host
- k_3 - mortality of 2nd and 3rd instar larvae within and when moving between hosts
- k_4 - pupal mortality in soil
- k_0 - variation in fecundity from potential maximum

Using techniques developed by Varley and Gradwell (1960), they concluded that the key-factor causing population change was k_0 , that this was density-dependent with a time delay, and that it was probably due to adult emigration and immigration. They found that larval survival was dependent upon egg density and shoot density, but less important than k_0 . However, they were unable to consider k_3 separately, since Bardner *et al.* (1973) had recorded numbers of pupae in only two years out of 11. It is this mortality factor which Ryan (1973b) found most important. Thus the question remains open as to whether k_3 might in fact be the most important mortality factor, and density-dependent, at least in some years.

Certainly WBF's habit of laying eggs in bare soil can be at least partially explained in terms of avoiding generalist predators, especially carabid beetles, which are more likely to be found in dense couch sod (Lagerlöf & Wallin, 1993) than in bare soil (Ryan 1973b).

The possibilities that k_2 , k_3 , and k_0 are density-dependent, and that each or all are most important in causing population change, are consistent with couch in a patchy environment as the natural habitat of WBF. Bardner and Fletcher (1973) found that, apart from lower egg numbers in the vicinity of vegetation, WBF eggs were evenly distributed in bare soil within a field. Thus k_2 should, perhaps, be considered separately in two different locations and on two different scales. On the local scale, in the zone at the edge of the hypsotactic shadow cast by mature couch plants, k_2 would be relatively low, since neonate larvae would be more likely to find suitable couch shoots for invasion in January. However, on the larger scale, beyond this zone, k_2 would still be very high. But in evolutionary terms, such high mortality would be an acceptable risk, because it would be accompanied by a small but significant chance of WBF larvae colonising a new patch of couch, and thus avoiding local extinction in a small and risky patch. Furthermore, in natural situations couch would probably grow in small, irregular patches interspersed with small, irregular patches of bare soil, and thus the proportion of unsuitable sites for larval feeding would be much lower than in the artificial situation of large bare or fallow fields.

Dempster and Pollard (1981) suggested that density dependence in WBF larvae only occurred when resources, i.e. wheat shoots, were limited. This would imply that WBF is an r-strategist, not a K-strategist.

Immigration or emigration of adults (k_0) can be seen in the same light. If k_0 is, as Kowalski and Benson (1978) conclude, density-dependent, immigration or emigration of adults would tend to compensate for patchy population densities in a patchy environment.

Of course, as Ray and Hastings (1996) state, the detection of density dependence in patchy population densities and patchy environments depends on the spatial scale under consideration. Taking their data from Kowalski and Benson (1978), they wrongly assume that WBF is monophagous, and that host plant distribution is uniform. However, by failing to take into account the biology of WBF, they fail to

realise that WBF can never have a spatially homogenous population density, whatever the scale. If couch is the natural host of WBF, and if oviposition is in bare soil, the population must be spatially heterogeneous.

Nevertheless, Ray and Hastings (1996) are correct in stating that, to detect density dependence, an appropriate spatial scale should be used. This scale may even be different for different stages of the insect's life cycle. Perhaps the spatial scale should be defined by the maximum distance the insect can move, i.e. 45cm for WBF larvae (Ryan, 1973b), and 0.8km for adults (Legowski *et al.*, 1968).

8.2.3. Development of couch patches and associated WBF populations

From the above, it can be inferred that WBF would favour a patchy habitat where couch can rapidly establish, but where there is still plenty of bare soil. Palmer and Sagar (1963) describe couch as a “pioneer plant in the colonization of waste places, when it tends to form pure stands, particularly if left undisturbed”; just such a habitat.

The life cycle of WBF can be explained in terms of the “habitat templet [*sic*]” proposed by Southwood (1977), and modified by Greenslade (1983) (Southwood 1988). (Fig. 8.2). The margins of a couch clump, with plenty of young shoots in which WBF larvae can develop, would be located in the top left corner of the diagram; a reasonably permanent, favourable habitat. The bare soil in which WBF eggs are laid would be located along the bottom edge of the diagram; a temporary habitat, more or less adverse depending on the numbers of couch shoots which actually appear when the eggs hatch in early spring. Thus the developing larvae are K-strategists, and the egg-laying females are r-strategists or A (adversity)-strategists, as defined by Greenslade (1983).

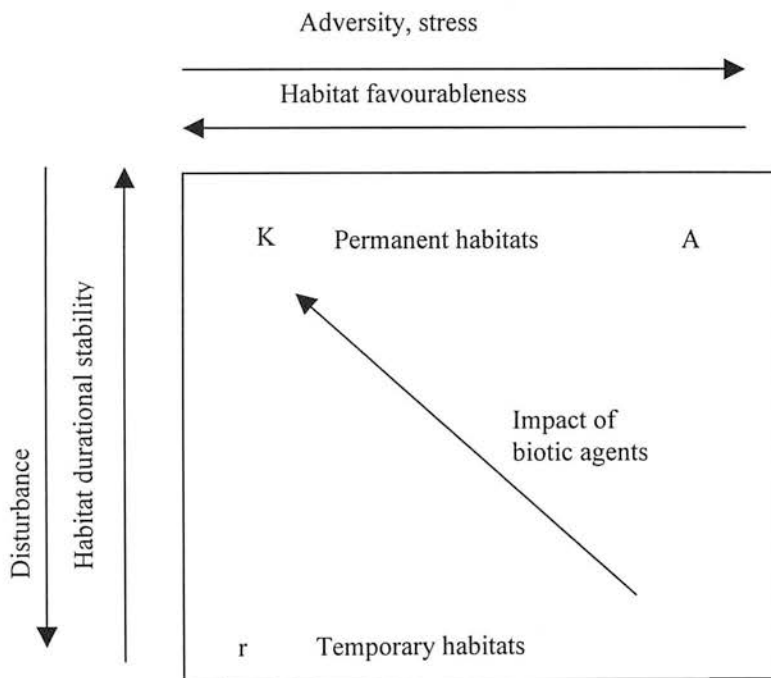


Fig. 8.2. Southwood - Greenslade habitat templet

Solbreck (1978) predicted several life-history responses of herbivorous insects to spatial and temporal change in the favourableness of resources in patches “here” and “elsewhere” (Fig. 8.3). Where changes in the favourableness of patches here and elsewhere are similar (Fig. 8.3a), better alternatives are not available, so diapause is favoured when the quality of all patches is low, as is the case with overwintering WBF. When the favourableness of patches elsewhere improves (or in the case of WBF, might be expected to improve) (Fig. 8.3b), migration should be favoured.

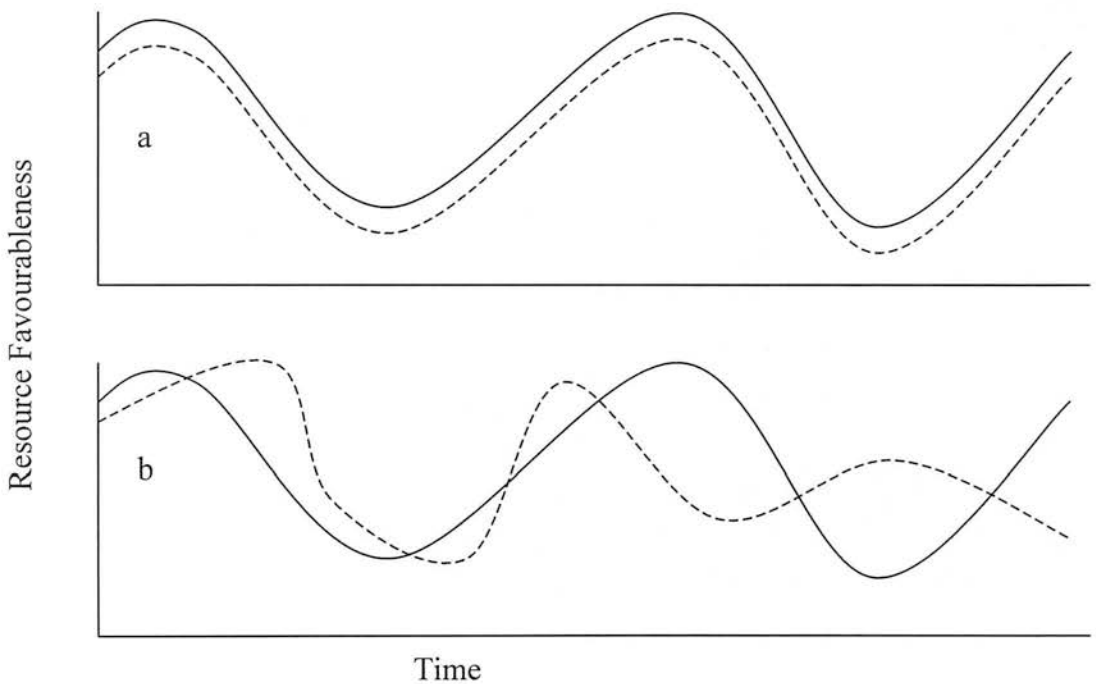


Fig. 8.3. Spatial & temporal changes in habitat favourableness. After Solbreck (1978)

- a. Changes “here” (—) and “elsewhere” (- - - - -) similar
- b. Changes “here” and “elsewhere” different

Brown and Southwood (1987) found such patchy habitats in the ruderal and early-successional types of plant community colonising bare soil. They described ruderal communities as “typically the first year of succession when annuals dominate”, although perennial grasses may also become established, and early-successional communities as “[typically] the second to fifth year where annual and biennial herbs

are declining but perennials and grasses are establishing". Fig. 8.4, adapted from Brown and Southwood (1987) illustrates these characteristics; note that from years one to three there is an extremely rapid increase in perennial grasses. This would be the most favourable period for WBF infestation of couch.

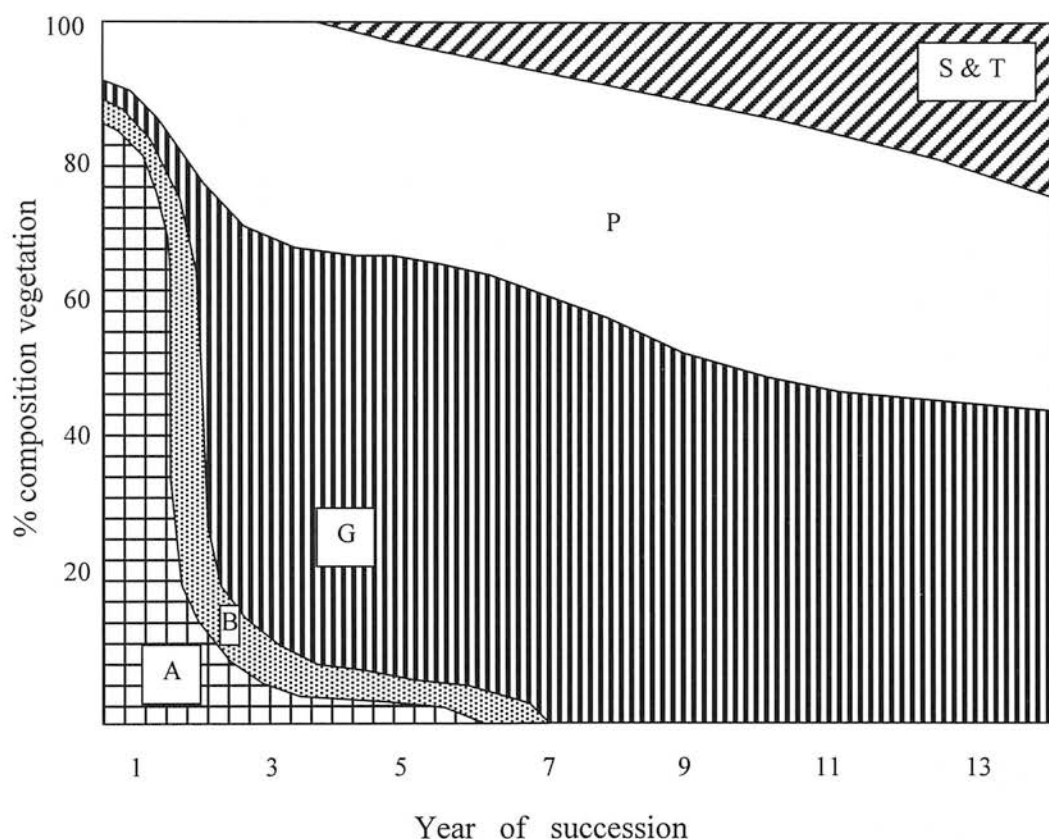


Fig. 8.4. Model of vegetation change in the same sites in terms of plant growth form

(After Brown & Southwood, 1987)

A = annual herbs & grasses, B = biennials, G = perennial grasses, P = perennials,

S & T = shrubs & trees

Hendrix, Brown and Gange (1988) found that herbivory by insects can begin to slow the rate of succession as early as the second year because of their impact on perennial grasses. Thus if WBF has a serious impact on the growth of couch, it could help maintain an appropriate habitat for longer. It would be possible to test the impact of

WBF on the rate of spread of couch clumps, using known numbers of larvae and an experimental design similar to that of Marshall (1990) in an area enclosed to prevent natural WBF oviposition. Hendrix *et al*'s methods (1988) could be repeated in the context of couch invasion of bare soil in an area subject to WBF attack, but it would be difficult, if not impossible, to attribute any slowing of the rate of succession to any one insect species, such as WBF.

Brown and Southwood (1987) also found a rapid increase in the numbers of phytophagous and, especially, predacious insects during the ruderal and early-successional stages of succession. The ratio of predators to phytophages was approximately 0.33 in the ruderal community, and 0.75 in the early-successional community. This lends further weight to the importance of predator avoidance in the behaviour and life-history of WBF.

Denno (1983) describes the ability of a plant-hopper, *Prokelisia marginata* (Van Duzee) to "track" its perennial grass host *Spartina alterniflora* (Lois.) in a patchy environment. *P. marginata* has a macropterous morph which migrates to new host patches, and a brachypterous morph which is more common on established hosts. WBF achieves the same results by different means. Genetic variation of WBF has never been described, and such crude polymorphism has never been observed. Instead, the gravid females migrate from the parent host to lay their eggs in bare soil, apparently without any host-seeking behaviour, and the larvae find host plants only on a very localised scale.

8.2.4. Evolution of WBF within a disturbed, patchy environment

There are several hypotheses, subject to considerable debate (Schoonhoven, Jermy and van Loon, 1998), which attempt to explain how oligophagous or monophagous insect herbivores become specialised on their hosts. Ehrlich and Raven (1964) proposed a process of coevolution, by which plants evolved defences against herbivores, which selected for herbivore behaviour to overcome those defences, which in turn selected for new herbivore defences. Bernays and Graham (1988) suggested that selection pressure from generalist predators on herbivores was more important in the evolution of host plant specialisation, while Fox (1988) proposed a more general hypothesis of diffuse coevolution, by which whole communities coevolved. Jermy (1984, 1988, 1993) has proposed “sequential evolution”, by which insect herbivores become more specialised in response to changes in their host plants, but exert little or no selection pressure upon them. The relevance of these hypotheses to WBF is discussed below.

For coevolution in the strictest sense to apply, it must be demonstrated that WBF and its host(s) exhibit “reciprocal selective responses” (Ehrlich & Raven, 1964) to each other. The present study has shown that WBF larvae respond preferentially to couch shoots, and that, in extreme circumstances, WBF infestation can affect the performance of couch rhizomes. But this adverse effect cannot be extrapolated to a population level, because adjacent nodes on a rhizome will compensate for death of terminal shoots, and even if couch rhizomes are killed, their neighbours can also compensate.

The effects of WBF herbivory on couch in the field are unknown, and even the appropriate methods to measure these effects have only rarely been used (e.g. Whitford, Rapport & de Soyza, 1999). Most studies on the effect of insect herbivores on their host plants (e.g. Agarwal, 1998) have taken production of seeds or other

reproductive bodies as a measure of plant fitness, but this is clearly inappropriate in a perennial grass which mostly reproduces vegetatively. Whitford *et al.* (1999) used the re-establishment (i.e. the rate of spread) of a perennial grass after a drought as a measure of “fitness”. This would appear to be a suitable measure of couch fitness in the face of herbivory by WBF.

Brown and Allen (1989) stress the importance of appropriate measurements when considering the impact of herbivores on food plants, and the ability of plants to compensate, or perhaps even over-compensate for negative impacts. In the present case, one could consider the number of couch shoots killed per unit area, the number killed per unit area of couch, or the proportion of couch shoots killed. The first two measurements would reflect the size of couch patches, and their coverage of the total area. Only the third measurement would reflect the impact of WBF on the ability of couch patches to spread further, and hence on the couch population. Fagan and Bishop (2000) give an example of the considerable effects insect herbivores can have on the spread of a perennial plant into bare soil.

Brown and Allen (1989) also point out that one should not assume a linear response of plants to herbivory; for instance, a plant might respond to damage by an initial surge in the growth rate, which diminishes after a while. Thus the times at which plant responses are measured play a crucial rôle.

Clearly, the pot trials in the current study provide only limited information on the spatial and temporal impact of WBF on wheat seedlings or individual couch plants with minimal resources, and to a lesser extent, on small communities of these plants. It would be unwise to extrapolate these results to make any conclusions about the impact of WBF on a couch community. However, even if this impact is high, it would be even more unwise to conclude, as coevolution would imply, that WBF is a

more important source of selection pressure on couch than any other biotic or environmental factor. Or, to quote Strong (1988): “Single factor explanations and simple dichotomies do not stand up to the evidence; no single factor along the gamut from plant chemistry to abiotic influences can be ruled out for even an interesting minority of cases; a complex of influences participate in the coaction of herb and herbivore.”

The evolution of oligophagy or monophagy as a response to generalist predators, as proposed by Bernays and Graham (1988), seems more plausible. Certainly WBF’s habit of laying eggs in bare soil can be at least partially explained in terms of avoiding generalist predators, especially carabid beetles, which are more likely to be found in dense couch sod (Lagerlöf & Wallin, 1993) than in bare soil (Ryan 1973a). This behaviour may provide a temporal as well as a spatial escape from predators, since Kruess and Tschardtke (1994) found that insect herbivores could colonise fragmented habitats before their natural enemies. And the habit of stem boring is likely to be due as much to defence against predators as to food choice. The meristem of a vigorously growing grass provides large amounts of suitable plant material with a relatively high proportion of proteins, but only by remaining within the shoot can WBF larvae avoid predators (Bernays, 1998).

Sequential evolution would be the most acceptable hypothesis if hydroxamic acids do indeed attract WBF larvae. In this scenario couch, wheat and other Triticeae evolved high levels of DIMBOA as a general defence against insect and microbial attack, then WBF overcame this defence by using DIMBOA as an attractant. Indeed, Menken (1996) and van Loon (1996) have proposed a mechanism by which this might occur. Using ermine moths of the genus *Yponomeuta* as an example, they suggest that insect sensory receptors respond to most secondary plant metabolites as deterrents, but that a small mutation might “switch” this deterrent effect to attractancy.

8.3. Possible future research

8.3.1. Further research on WBF's relationships with host plants

The present study investigated only a few, very limited, aspects of the relationship between WBF and couch.

Larval bioassays showed that neonate larvae were more attracted to couch seedlings and their exudates than to wheat seedlings and their exudates. Although a strong attraction to couch rhizome exudates was demonstrated, responses to these exudates were not directly compared to responses to couch seedling exudates or to wheat seedling exudates. This merits further study, since neonate WBF larvae are more likely to encounter couch rhizomes than couch seedlings (Holm *et al.*, 1977).

The present larval bioassays investigated geotaxis, phototaxis, and response to host plants or their exudates separately. Further study on interactions between these responses could establish whether WBF larvae find their hosts through a catenary process, and could provide valuable information on the optimal placement of insecticides.

The relationship between developing WBF larvae and host plants was measured only in terms of WBF mortality and time to eclosion, and the number of host shoots, leaves, and deadhearts. No attempt was made to weigh either the insect or the host at any stage of their development, yet plant biomass and resource partitioning, and insect pupal weight may be important measures of fitness. Measuring these parameters would increase our understanding of the relationship between individual insects and plants in the period until the eclosion of WBF adults.

Longer-term and larger-scale field trials could increase our understanding of the relationship between populations of WBF and couch. Marshall (1990) showed that the tall oat-grass *Arrhenatherum elatius* (L.) Beauv. ex J. & C. Presl, and to a lesser extent, other grasses, could limit the rhizomatous spread of couch grass. It should be

possible, using similar methods, to investigate the effect of WBF larvae on the spread of couch patches.

8.3.2. Implications for pest management of WBF

As indicated in Chapter 1, there are several problems with current management of WBF. The current findings provide several indications as to how future research might improve WBF pest management.

The insect does not cause significant problems every year, and chemical control once damage is seen may be difficult and too late, so farmers rely on predictions of WBF population levels to determine whether control is necessary. Counts of WBF eggs from likely oviposition sites can give a good indication of the numbers of larvae likely to hatch in January and February (Raw, 1967). However, current methods of extracting eggs from soil samples are very time-consuming, so advisory bodies cannot at present conduct egg counts on every field at risk, yet even in high-risk areas, egg counts can vary greatly from field to field (K. Kasperek, pers. comm.). Thus, at present, risk prediction is based on a small number of egg counts in sample fields, and/or predictions of egg numbers from a model based on meteorological data (Young & Cochrane, 1993).

Predictions of egg numbers could be improved if the numbers of gravid female WBF could be reliably assessed. However, the present research gave no indication that gravid female WBF respond to olfactory cues, so there is little hope of developing traps for assessing numbers of gravid females.

Egg counts remain the best available predictor of larval populations, so an improvement in the method of sampling and extracting eggs from soil could allow egg counts to be conducted on a farm-by-farm basis. The technology, based on washing soil through sieves and floating eggs in saturated magnesium sulphate solution, is simple and of minimal risk to the operator. In SAC's Standard Operating

Procedure SE020 (SAC, 2000; see Appendix 1) 24 soil samples are taken with a standard-sized shovel from across the longest diagonal of the field; this process itself is time-consuming. It takes at least one hour to process an ordinary soil sample and to count the eggs, mainly because the soil structure makes the sample hard to break down, and the eggs have to be separated from any other organic matter in the soil (pers. obs.) However, the trays of sand used in the oviposition trials described in Chapter 7 could be processed in half the time. If egg numbers could be assessed by placing trays of sand in likely oviposition sites, the time taken for both collection and processing of samples could be much reduced. The method has changed little since it was first developed by Salt and Hollick (1944), so with research by engineers it may even be possible to produce a faster and/or smaller-scale method of egg extraction which could be used by farmers. This would be ideal, as even substantial improvements in soil sampling and laboratory-based processing of soil samples would not allow advisory bodies to undertake egg counts for every farm at risk.

Oakley and Uncles (1977) found that, by using soil in oviposition trays, they could predict final numbers of eggs by counting eggs until 20th August, and thus advise farmers of likely egg numbers well in advance of sowing autumn cereals. Using sand instead of soil in oviposition trays, and an improved, small-scale farm-based method of extracting and counting eggs, could allow field-by-field predictions of egg numbers.

The risk of WBF attack is currently assessed by taking account of actual or forecast egg numbers, previous cropping, sowing date of the crop, plant population and growth stage, and previous history of damage on the farm (HGCA, 2000). This last factor is very significant, since it is well known that some farms or areas have a long history of high levels of WBF damage (K.A. Evans, pers. comm.), but the reasons for these high risk areas have only rarely been considered. Gough (1949) found very low levels of WBF eggs in some areas of Yorkshire where climate and previous cropping patterns might be expected to favour the insect, and Long (1958b) suggested that this,

and similar examples at Rothamsted, might be due to the absence of a nearby source of adult WBF.

If couch grass is the preferred host of WBF, this raises the interesting question of whether patches of couch near wheat act as a “decoy” diverting the pest from wheat, or as a “reservoir” from which the pest can spread into wheat. There is very scant evidence for either hypothesis. However, Legowski *et al.* (1968) and Oakley (1980) both achieved some success in reducing WBF populations within a large area either by not sowing winter wheat (Legowski *et al.*, 1968), or by controlling WBF with pesticides (Oakley, 1980) within that area. Conversely, the farms in East Lothian, covering an area of approximately 2 x 2.5km, where the current field studies were undertaken continue to have high populations of WBF, despite routine applications of chemical controls, usually seed treatments, for the last 30 years (Pers. obs., W.D. Simpson & Son, pers. comm.). This suggests that within the area of these farms there exists a “reservoir” of WBF; and, indeed, right in the centre is a rough grass-covered knoll where couch is one of the principal species. This could explain why WBF damage persists on these farms, despite routine control measures. Thus the available evidence supports the “reservoir” hypothesis.

Molecular markers, as suggested by Loxdale and Lushai (1999), could be used to investigate the dispersal of WBF populations and metapopulations, and to test the “decoy” and “reservoir” hypotheses. If related to possible sources of adult WBF from detailed maps of couch clumps and other WBF hosts, such research might help predict the risk of WBF attack.

Current chemical controls of WBF rely mainly on killing larvae, preferably neonates, outside the host plant. This is attempted either with a seed treatment of tefluthrin, a synthetic pyrethroid, or with organophosphates applied at the time of egg-hatch, or as a last resort, when damage is first seen. High concentrations of the chemical will only occur near treated seeds, or near the surface of the soil; larvae which avoid these areas will escape control (Young & Ellis, 1996). The present research indicates that

neonate larvae would remain below the soil surface, thus avoiding soil-applied insecticides. Previous research (Long, 1958a), indicates that they are attracted to the lower stem, or bulb, of wheat shoots, thus avoiding seed treatment insecticides. Prompt application of post-emergence insecticides, if needed, is very important, but this is not always possible in January and February due to adverse soil and weather conditions. So, although the chemicals used are highly toxic to WBF larvae, they cannot always be delivered to the insect at the time and place when it is most vulnerable.

As mentioned in Chapter 1, many chemicals used to control WBF are organophosphates, a class of insecticide which is causing increasing concern over possible adverse effects on human health (Marrs, 2000) and the environment (Burn, 2000). Approval for use of these products may soon be withdrawn (Young & Ellis, 1996), so new alternative methods of control would be very desirable.

Thus new methods of WBF control should rely on a more site-specific prediction of risk, should be more reliable in their delivery to neonate or first instar larvae, and should reduce or replace the use of organophosphates. The most cost-effective method of control is seed treatment (HGCA, 2000).

The most effective method of reducing damage by WBF is to prevent larvae entering the cereal shoot. The responses of neonate WBF larvae in the present choice test bioassays (Chapter 3), and in previous research (Scott & Greenway, 1973, 1976) suggest semiochemicals could play a part in this. Attractants isolated from couch, and arrestants isolated from wheat, could be used to draw neonate larvae away from cereal shoots, and/or towards lethal concentrations of insecticide. Extracts of oat seedlings were found to repel WBF larvae (Scott & Greenway, 1976), and when applied to soil reduced the numbers of infested wheat shoots 28 days after WBF eggs were added (Scott & Greenway, 1973). From these results, they concluded that oat extracts had some toxic or deterrent effect on WBF larvae. Thus, semiochemical(s) isolated from oats could also be used to deter WBF larvae from wheat seedlings.

The isolation and identification of these semiochemicals would probably be a lengthy process, similar to that undertaken by Greenway, Scott, Calam and Smith (1976). However, an initial comparison of GC/MS or HPLC profiles of wheat, couch and oat exudates might yield interesting results, and, for reasons outlined in Chapter 3, the hydroxamic acids, especially DIMBOA, merit further investigation as possible attractants and arrestants of WBF larvae.

Once these semiochemicals have been identified, their effectiveness could be easily tested in choice test bioassays like those in the current study. Exactly how insecticide, attractant, arrestant and deterrent should be used, and in which combinations, would need substantial further research in the field and the glass-house. Possible uses are shown in Fig. 8.5, and are described in order of increasing complexity below. The simplest and most economical should be researched first; if these were found effective, research into more complex and costly uses of WBF semiochemicals would be unnecessary.

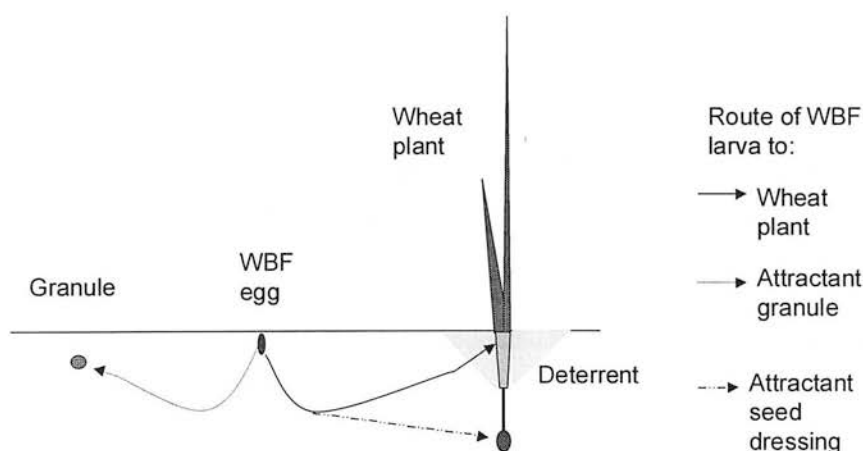


Fig. 8.5. Possible uses of semiochemicals in WBF control

The geotactic response of neonate larvae, as shown in the present research, suggests that, when larvae move upwards from their hatching site, they are simply responding to host plant attractant(s). So it may be possible to control larvae simply by adding a

stronger attractant, such as that found in couch, to current seed treatments, thereby drawing larvae away from the cereal bulb, and into a lethal concentration of insecticide. If this were effective, it may be possible to reduce the dosage of insecticidal seed treatment.

However, it may be necessary to draw neonate larvae away from the cereal seedling entirely. This would require incorporation of attractant and arrestant into a granule, which should be buried to exploit the geotactic and photophobic responses of neonate larvae demonstrated in the present research. A separate operation to incorporate such granules into the soil before sowing would probably be prohibitively expensive, as was the similar use of chlorpyrifos (Young & Ellis, 1996) which has now been discontinued. Adding granules to the seed drill might, however, be more economically viable. It might be necessary to incorporate an insecticide into such a granule, since attempts to “confuse” larvae with field applications of wheat exudate actually increased the numbers of larvae found, presumably by attracting larvae from outside the plots (Long, 1959). Research by Cockbain (1968), and by Cokmus and Elçin (1995) suggests that it might be possible to replace chemical insecticides in a slow-release granule with *Bacillus thuringiensis* var *israelensis*.

It might also be possible to adopt a “stimulo-deterrent diversionary strategy” (SDDS) (Miller & Cowles, 1990; Pickett, Wadhams & Woodcock, 1995) by combining attractant granules with a deterrent dressing on the wheat seeds, or a deterrent spray around the wheat plant. SDDS has been successfully used in small-scale, tropical intercropping, in the management of stem borers and parasitic weeds for maize and sorghum in Africa (Khan, Pickett, van den Berg, Wadhams & Woodcock, 2000). Management of WBF could provide an excellent opportunity to demonstrate such a strategy in large-scale intensive arable farming in a temperate climate.

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Appendix 1

SAC Standard Operating Procedure SER 020.

Wheat bulb fly sampling and assessment procedures

Uncontrolled copy

Title	Wheat bulb fly sampling and assessment procedures				SOP CER 020
SOP Location		----	----	----	----
Amended	31 AUG 00	----	----	----	----
Effective Date	01 NOV 00	Issue Date	01 NOV 00	Version	two

Author Signature		Management Signature		QA Signature	
Date	__ __ __	Date	__ __ __	Date	__ __ __

Title : Wheat bulb fly sampling and assessment procedures

Objective: To estimate wheat bulb fly populations and the risk of damage

Field of Application: Winter wheat

Responsibility: Trained staff

Documentation Required: None

Check Divisional Risk Assessments before carrying out this procedure.

1. Procedure

1.1 Assessing the population of wheat bulb fly and the risk to a winter wheat crop, can be undertaken at two different timings; prior to sowing, where an assessment of the number of eggs/ha can be obtained by soil sampling, and/or in late February/early March, where an assessment of the number of wheat bulb fly larvae in wheat plants can be undertaken.

1.2 Assessing the number of wheat bulb fly eggs prior to sowing

Wheat bulb flies lay eggs on bare soil between July and late September. Consequently eggs tend to be laid in fields that have crops of potatoes, peas, oilseed rape or fallow set-aside. Sampling for wheat bulb fly eggs should take place before any soil cultivation as this buries the eggs and impairs the accuracy of the population estimate.

A shovel with side height of 3cm and width of 10cm is used to take soil samples to depth of 3cm and a distance of 7.5cm. This gives a soil sample of 0.0075m². Any shovel can be used providing the area of soil sampled is known.

In potato crops 24 samples should be taken along the longest diagonal of the field, 12 from the furrow and 12 from the ridge. Furrow and ridge samples should be bagged separately. In other crops, 24 samples should be taken across the longest diagonal.

Each sample of soil is washed through a Fenwick can, containing a 2mm sieve and the washings collected in a 355µm sieve. The contents of the 355µm sieve are washed into a funnel containing filter paper using saturated magnesium sulphate solution which causes the eggs to float. The eggs are removed with a fine camel hair brush or forceps and counted. The results are expressed as total numbers of eggs/ha based on a multiplication factor derived from the sampling method. In the case of 0.0075m² per sample, the multiplication is 10,000/(24 x 0.0075) = 55555.5. (See McKinlay & Franklin, 1980 for full details).

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1.3 Assessing level of wheat bulb fly infestation of wheat plants

In late February or early March, depending on the season and when egg hatch of wheat bulb fly has been recorded, (typically one month after the beginning of egg hatch), an assessment of the level of wheat bulb fly infestation can be carried out in the field by counting the number of plants which contain a wheat bulb fly larva. Infested plants will exhibit 'deadheart' symptoms which is a yellowing of the central shoot caused by the death of the shoot due to wheat bulb fly feeding. However a more accurate assessment of the level of wheat bulb fly infestation is to dissect plants and look for the wheat bulb fly grub. At least one hundred plants (roots as well) should be randomly sampled in a 'W' pattern across the field. The plants should be dissected in the laboratory and the numbers of wheat bulb fly grubs present recorded and expressed as a percentage of infested plants.

Reference

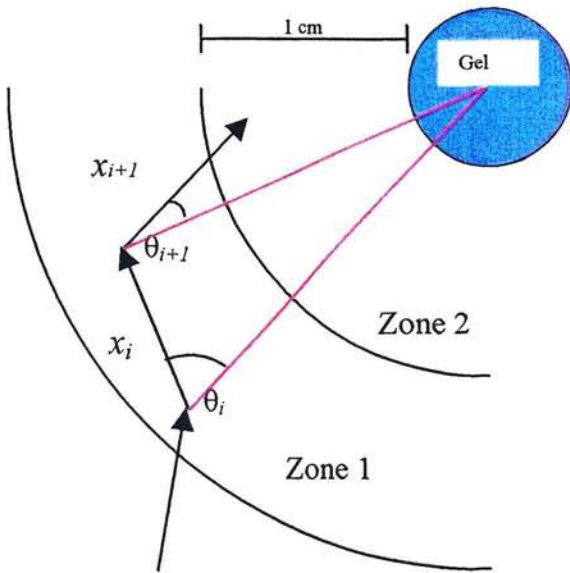
McKinlay, R.G. & Franklin, M.F. (1980). A comparison of a corer and a shovel for sampling populations of wheat bulb fly (*Delia coarctata*) eggs. *Annals of Applied Biology* **95**, 279-222.

END of SOP

Author Signature	Management Signature	QA Signature

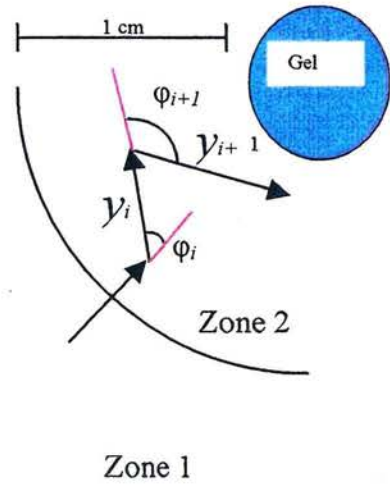
Appendix 2

**Calculation of attractancy and arrestancy
of gels and seedling exudates from wheat bulb fly
larval choice test bioassays: Worked example**



Calculation of attractancy parameter, S

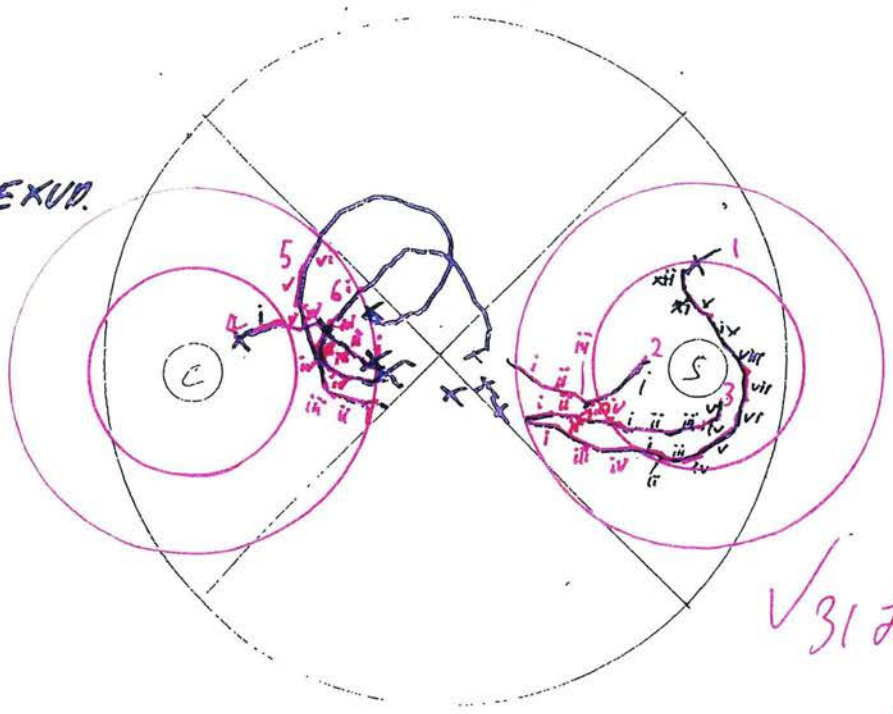
$$S = \sum x_i \theta_i / 180 \sum x_i$$



Calculation of arrestancy parameter, K

$$K = \sum \phi_i / \sum x_i$$

Date: 28 FEB 2000
 Time: 14.55
 Control = GEL
 Stimulus = COUGH GEL EXUD.
 n = 10
 Replicate = 13



✓ 31 Feb

4. Couch. Couch sdlig exud. vs. control (2)										4. Couch sdlig exud. vs. control (2)										4. Couch sdlig exud. vs. control (2)										4. Couch sdlig exud. vs. control (2)									
sdlig exu vs control		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date		Date			
Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep			
13		13		13		13		13		13		13		13		13		13		13		13		13		13		13		13		13		13		13			
x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x			
Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva		Larva			
1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19			
x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x		x			
1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C		12 C		13 C		14 C		15 C		16 C		17 C		18 C		19 C			
1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C		12 C		13 C		14 C		15 C		16 C		17 C		18 C		19 C			
1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C		12 C		13 C		14 C		15 C		16 C		17 C		18 C		19 C			
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1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C		12 C		13 C		14 C		15 C		16 C		17 C		18 C		19 C			
1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C		12 C		13 C		14 C		15 C		16 C		17 C		18 C		19 C			
1 S		2 S		3 S		4 C		5 C		6 C		7 C		8 C		9 C		10 C		11 C																			

Appendix 3

Choice of oviposition site by female wheat bulb flies:

Site details of field trial

SAC CROPS DIVISION SITE DATA SHEET		Page of
SAC Study No:-		Study director:
Title:-		
SITE MANAGER:-		YEAR: 2000

FARM NAME, ADDRESS AND FIELD NAME:-

NEWHOUSE FFM, NORTH BERWICK, E. LOTHIAN

NEWHOUSE LANE EAST

GRID REF:- NT 538830

ELEVATION:- 180 ft at

SOIL SERIES:-

SOIL TEXTURE:-

pH. 6.8

PREVIOUS CROPPING

1999 WHEAT

1997 WHEAT

1998 WHEAT

1996 WHEAT

DESIGN:- RANDOMISED

BLOCK

NUMBER OF REPS: 5

VARIETY:- MARIS PIPER

DATE SOWN:- 3 APRIL 2000

SEED RATE:- 3 kg/ha

	RATE/ha	PRODUCT	DATE	G.S.
HERBICIDE 1:	4 lt	CYANAZINE + PENDIMETHALIN	9 MAY 2000	
	1.5 lt	DICUAT + PARAQUAT		
HERBICIDE 2:	1.5 lt	DICUAT	4 SEPT 2000	
	2.5 lt	DICUAT	8 SEPT 2000	
FUNGICIDE 1:	2.5 kg + 0.1 lt	CYTOXANIL + MANCOZEB	27 JUNE 2000	
		+ OXADIXYL + ADJUVANT		
FUNGICIDE 2:	2.5 kg + 0.1 lt	CYTOXANIL + MANCOZEB	13 JULY 2000	
		+ OXADIXYL + ADJUVANT		
FUNGICIDE 3:	2.0 kg +	DITETHOCHLORPH + MANCOZEB	25 JULY 2000	
	0.1 lt	+ ADJUVANT		
FUNGICIDE 4:	0.3 lt	FLUAZINAM	11 AUG 2000	
FUNGICIDE 5:				
OTHER SPRAYS:	75 mls	LAMBDA-CYHALOTHIN	27 JUNE 2000	
	3.0 kg	METHIOCARB	27 JUNE 2000	
	3.0 kg	METHIOCARB	13 JULY 2000	
	3.0 kg	THIODICARB	25 JULY 2000	
	3.0 kg	THIODICARB	11 AUG 2000	

Comments:-

SEED

POTATOES

Signature:-

Charles Marriott